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Erratum

Vol. 70(2), 1954. *Comments on the teaching of trigonometry.* T. F. Hickerson. P. 229, third from last line: read "sines" for "cosines".

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SEASONAL HISTORY STUDIES ON THE SOUTHERN CORN ROOTWORM AND ITS RELATION TO PEANUT CULTURE IN NORTH CAROLINA[1]

By W. H. Long[2] and J. R. Dogger

North Carolina State College, Raleigh, North Carolina

Introduction.—There has been a growing need during the past few years for a more intimate knowledge of the seasonal history of the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, as it is related to peanut culture in North Carolina. The studies reported here were especially aimed at gathering information pertinent to the following questions: How many generations occur each season in peanut fields? When does infestation first occur? At what period during the growing season is most of the damage done? How important is this injury?

Literature.—Early records of life history studies of the twelve-spotted cucumber beetle were published by Garman (1891) and Riley (1891). Following these a number of original and comparatively sizeable contributions to our knowledge of the insect's life and seasonal history were made by the following authors: Quaintance (1900); Chittenden (1905); Marsh (1912); Thomas (1912); Webster (1913); Luginbill (1918); Sweetman (1926); Arant (1929); and Isely (1929). Most of this work resulted from economic injury to corn and vegetables by *Diabrotica*.

Almost forty years ago Fink (1916) reported injury to peanuts from the southern corn rootworm in Virginia and included pictures of rootworm-injured peanut pods. Grayson (1947), working at Holland, Virginia, reported on *Diabrotica* as a pest of peanuts. He included a discussion of injury, economic importance, factors affecting injury, laboratory and field control, phytotoxicity of DDT and BHC, and life history studies. Fronk (1950) conducted further studies at Holland, Virginia, on the cultural and biological control of the rootworm in peanuts. He was concerned with varietal resistance, plant spacings, planting dates, and a survey of the natural enemies of *Diabrotica*.

For North Carolina, no comprehensive studies have been made on the twelve-spotted cucumber beetle regarding its biology and depredations as they occur in the peanut fields. Sherman (1905, 1914) gives some opinions regarding the insect's

[1] Contribution from the Entomology Department. Published with the approval of the Director of Research as Paper No. 589 of the Journal Series.

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life history based on field observations in North Carolina. Smith (1908, 1910) and Metcalf (1909) have discussed this insect briefly while considering insect enemies of truck crops and tobacco, respectively. Fulton (1946) reported on control of the southern corn rootworm in corn with soil insecticides. Ritcher *et al.* (1952) published the results of some studies on insecticidal control of this insect in peanuts.

LIFE HISTORY.—Limited observations on the life history of the southern corn rootworm were made in the laboratory during 1952, 1953 and 1954. These observations indicate that completion of the life cycle from egg to egg requires an average of about six weeks, but may vary from four to nine weeks, depending largely upon the time of year as it affects existing temperatures. Further indications are that optimum temperatures for rapid development are from seventy-nine to eighty-two degrees Fahrenheit for most of the immature stages. The approximate length in days for each developmental period averaged as follows: egg, seven; feeding larva, twelve; prepupa, five; pupa, seven; and preoviposition period, ten. There is considerable divergence of opinion in the literature regarding the length of the oviposition period. Webster (1913) states that there is a tendency for the individual female to complete oviposition within a few days. Quaintance (1900) believed that a beetle would normally deposit its eggs in the course of one or two days. Isely (1929) reports an oviposition period of forty-two days for eight first brood females. Arant (1929) states that the average duration of this period for overwintered beetles was forty-three days; for first generation beetles, eighteen days; and for second generation beetles, ten days. Observations made by the authors indicate that an individual beetle in nature may deposit eggs for a period of six to eight weeks and perhaps longer.

SEASONAL HISTORY.—The data for seasonal history studies reported on below were gathered from overwintering cages and systematic field observation and sampling. Two light traps were also maintained and operated at weekly intervals, at Rocky Mount and Lewiston, N. C. respectively. These traps were modeled after the type used by Hiestand (1928) with only slight modifications. It is hoped that the information gathered from these several sources, supplemented by the limited life history studies reported above, will give us a clearer conception of the phenomena relating to the life of this insect as they occur throughout the season.

Overwintering.—One hundred beetles were caged in the field during September and October, 1953, to determine whether or not they could survive the winter in North Carolina. Fifty beetles, collected from rose and dahlia blooms near Raleigh, were confined in each of two cages on September 28 and October 14, respectively. These wooden-framed cages were rectangular in shape, 24 inches long by 18 inches wide by 18 inches high, and covered with screen wire. The bottoms were open and one side was equipped with a hinged door, 9½ by 11½ inches, that latched. The bottom of each cage was planted from one to two inches below the ground level with soil packed firmly around the sides, to prevent any beetles from escaping. Both cages were placed over common broomsedge, the first one at the edge of an apple and peach orchard adjacent to woods, and the

TABLE 1

Number of beetles surviving the winter of 1953–1954 in outdoor cages at McCullers, N. C.

Cage No.	No. of Beetles Confined	Date of Confinement	Date of Cage Opening	No. Live Beetles Recovered	Total Days of Confinement	Per cent Beetles Surviving
1	50	Sept. 28	Mar. 10	7	163	14
2	50	Oct. 14	Mar. 24	12	161	24
Totals	100			19		19

second in a low sheltered area with woods nearby. Bissell (1939) reported from Georgia that broomsedge seemed to be by far the favorite winter quarters of the cucumber beetle. The cages were situated at McCullers Experiment Station, about twelve miles south of Raleigh on highway 15A.

Both overwintering cages were visited on November 20 and active beetles were observed in each. The cages were opened during March, 1954, and the results obtained are shown in Table 1. Nineteen percent of 100 beetles confined in these outdoor cages survived the winter during more than 160 days of confinement. The winter of 1953–1954 was not unusually mild for North Carolina, so it seems probable that many beetles normally overwinter in the state. Sex ratios were not determined for the 100 beetles originally caged, but on the basis of the known ratios for beetles taken in the field during the late summer, it seems probable that at least half of them may have been males. Eighteen of the nineteen living beetles recovered from the cages in March were known to be females; the remaining one accidentally escaped before its sex was determined. Sell (1916) states that females are heartier than males. Arant (1929) reports that females are more vigorous, live longer and that a higher percentage live over winter than males. Eggs were first deposited by some of the overwintering females seven days after they were brought in from the field and placed in lamp chimney cages in the laboratory. These eggs hatched after an incubation period of eight or nine days.

Seasonal Population in Peanut Fields.—Systematic field observations were conducted during 1953 from May 21 to September 25 at the Upper Coastal Plain Experiment Station, Rocky Mount, N. C., and at the Peanut Experiment Farm, Lewiston, N. C. Lewiston is located approximately in the heart of North Carolina's peanut-growing area, while Rocky Mount might be considered close to the southwestern edge of this area. The fields under observation at these two stations were planted May 6 and April 27, respectively. Soil examinations were made at each station at approximately ten-day intervals. At each examination an ordinary spading fork was employed in turning and examining samples of a single foot of row. Each foot sample reached to the middle on either side of the peanut row and was approximately eight to ten inches deep. The soil was examined by hand on the spot, and all pods showing possible injury were opened in an attempt to find insects that might have been feeding within. All rootworms that could be found were brought back to the laboratory in 2½ inch salve boxes, containing the soil in which they were taken, for determination of instars and further rearing. Counts of adult beetles were also made in the area of each soil

sample by stooping or crawling along ten feet of row and recording the total numbers of beetles taken and seen. This method progressively required more time as the season advanced and the plants became larger. In spite of this, the crawling technique was considered superior to sweeping as it was believed to give a more accurate record of the actual numbers of beetles present in any particular sample, and also as it was not accompanied by the bruising or otherwise injuring of plants that often accompanies sweeping. At Lewiston one sample was taken from each of thirty-six untreated check plots. At Rocky Mount, two samples were taken from each of twenty-four plots, twenty-one of which had received various insecticidal soil treatments before the first cultivation. Plants were from three to four inches high at each station when the first samples of the season were taken.

During the entire season a total of only 266 beetles, 143 larvae and twenty-six pupae were taken in plot samples at both stations. A summary of these data from both stations is presented in Table 2, and is thought to be a more accurate representation of population conditions throughout the peanut growing area than similar presentations for the separate stations would have been, owing to the seemingly small number of the insect taken in samples. The past two years have been unusually dry for North Carolina, however, and wetter years might result in observations differing somewhat from those reported here.

Table 2 indicates that twelve-spotted cucumber beetles were continually present in peanut fields from early June through September, or until harvest which came during late September and early October at both stations. Beetles were apparently most abundant in peanut plots during mid-August; however,

TABLE 2

Total numbers of Diabrotica undecimpunctata howardi *counted in peanut plots at Rocky Mount and Lewiston, N. C., during the 1953 season*

Period*	Larvae	Pupae	Adults
May III†	2	0	0
June I	0	0	16
June II	0	0	18
June III	1	0	13
July I	3	0	12
July II	4	0	19
July III	3	1	33
Aug. I	12	2	36
Aug. II	16	3	45
Aug. III	54	12	25
Sept. I	24	3	19
Sept. II	22	3	18
Sept. III†	2	2	12
Totals	143	26	266

* Roman numerals I, II, and III represent the first, second, and third ten days of each month, respectively.

† Represents Rocky Mount only; no counts made at Lewiston for this period.

this should not necessarily be interpreted to mean that there are more southern corn rootworm adults in August than at any other time. There was more soil moisture in these fields about mid-August than there was at any other time during the season. Following this the soil became progressively drier at both stations until harvest. During September, peanut plants were less succulent and were not as green as they had been previously. It is possible that many beetles sought their sustenance elsewhere. Large numbers of beetles were observed feeding in rose and dahlia blooms at Raleigh during late September. Although beetles were observed to feed on both foliage and flowers of the peanut during the season, the flower may hold some greater attraction for them. Smith (1910) reports that these beetles may continue feeding in cucurbit blooms during the entire season of growth, also stating that they feed on the flowers of field crops and fruit trees and might be termed omnivorous and omnipresent.

Southern corn rootworms were found continuously in peanut plots from late June until harvest. These larval populations tended to increase gradually to a high peak in late August, which was followed by a slow decline through mid-September. A comparatively much smaller number of pupae was observed during the season than adults or larvae, but here again a distinct population peak is apparent in late August. This comparative scarcity of observed pupae might be attributed to their habit of pupating in earthen cells, numbers of which could have been missed during the sampling process in spite of all attempts to avoid this.

Seasonal Oviposition.—Many of the females collected during 1953–1954 were dissected to determine the numbers containing fully developed ova (hence probably ovipositing) at different times of the year. Table 3 indicates that eggs were being deposited in the peanut plots from June through August. Some eggs were apparently deposited during early September, but for all practical purposes these should be considered as insignificant. The first observation of active beetles in the field in 1954 was during the last week of March. At this time they appeared to be fairly common in alfalfa on warm days, and, as Table 3 indicates, all of the observed beetles were gravid. Of the females collected in April, 56.5

TABLE 3

Total number of female beetles dissected and the percentage of gravid females during months of 1953–1954

Period	Total Numbers of Females	Percentage of Total that were Gravid
June, '53	33	22.7
July, '53	238	32.1
August, '53	170	31.2
September, '53	69	2.9
October, '53	15	0.0
November, '53	15	0.0
Late March, '54	14	100.00
April, '54	23	56.5
May, '54	10	30.0

percent contained fully developed ova, but numerous young ova were apparent in the ovarioles of many of the rest. Observations by Sherman (1914) from 1904 to 1913 indicate that the first active beetles of the year generally appear sometime during the last three weeks of March in the vicinity of Raleigh, N. C. Grayson (1947) states that during 1945–1946 at Holland, Virginia, eggs were deposited in a screened insectary by the overwintering beetles as early as the third week in March. Thomas (1912) reports from South Carolina that the first eggs of the year were laid on March 10, and that fully developed beetles from these eggs had all emerged by May 24.

Discussion.—As has been shown in Table 2, twelve-spotted cucumber beetles first appeared in the peanut plots in early June. Observed beetle populations during the spring of 1954 tend to support this observation, although plant development in early June, 1954 was considerably behind that for the same period of 1953.

A sufficient population of rootworms was observed in peanut plots at Rocky Mount during late May, 1954 to indicate that the larval population recorded for the same period of 1953 (Table 2) is probably the rule rather than the exception. These are insects of the first generation and are almost certainly present as either eggs or young larvae before peanuts are planted. Table 2 further indicates the absence of larvae during most of June, their presence not having been detected again until late June and early July. No rootworms at all were found in peanut plots during June, 1954. Larvae appearing in late June and early July are considered to be of the second generation and come from eggs laid by beetles present in the peanut plots after the plants have sprouted and growth has gotten underway. After this there is ample time for and most certainly is a third generation, but owing to the quite extensive oviposition period of this insect, these second and third generations overlap so that their separation is not possible in the field.

NATURE AND EXTENT OF INJURY.—Although twelve-spotted cucumber beetles feed upon the foliage and flowers of the peanut plant, injury inflicted in this way is of very little importance in comparison with that caused by the larvae. Rootworm injury may easily be confused with that of the larva of the elongate flea beetle, *Systena elongata*, especially where first and second instar rootworms are responsible (Long and Dogger, 1953). The larvae bore through the shell and into the pods, seeming to prefer the younger, immature pods. Injury to more nearly mature pods also occurs, although these pods usually show only pits and scars denoting attempted entrance. After penetrating the shell, the insect tunnels and feeds in the soft, succulent, inner tissues of the pod and in the developing kernels themselves. In cases where insect tunneling has not resulted in complete economic loss of the pod, the tunnels afford easy entrance for decay organisms which quickly render the pod worthless.

Table 4 shows the estimated amount of injury to peanut pods inflicted by the southern corn rootworm, together with the total amount of insect injury occurring during the 1953 season in peanut plots at Lewiston, N. C. Although the first rootworm injury was observed on July 9, the greatest injury occurred during

TABLE 4

Insect injury to peanut pods at the Peanut Experiment Farm, Lewiston, N. C., 1953

Date	No. of Plants Examined	Av. No. of Pods per Plant	Per cent of Pods Injured by *Diabrotica*	Total Pods Injured
				per cent
June 30–July 1	51	1.1	0.0	0.0
July 9	53	6.6	0.9	1.2
July 20, 21	50	13.0	4.3	4.3
July 30, 31	50	22.5	2.8	3.0
August 7, 8	42	38.3	1.9	5.1
August 18, 19	50	47.5	1.9	7.0
August 26, 27	106	47.3	7.9	12.7
September 7, 8	58	54.2	8.9	11.6
September 17, 18	43	57.0	13.3	15.3

August and September. Other insects were responsible for damage; however, it is believed that in North Carolina the rootworm larva is responsible for more damage to peanut pods than any other insect. Ritcher *et al.* (1952) reported as much as fifty per cent of the pods injured in untreated plots by harvest time in a field at Rocky Mount, N. C. The same authors observed, however, that some of the injury in this field was caused by wireworms of the genus *Melanotus* and larvae of the elongate flea beetle, *Systena elongata*.

SUMMARY

Available information indicates that the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, is probably the most important subterranean pest of peanuts in North Carolina.

There are three complete generations per year in North Carolina, the second and third inflicting losses in peanut fields.

Owing to a variable but extensive oviposition period, the second and third generations overlap so that their separation is not possible in the field.

Laboratory rearing indicates that the average time required to complete the life cycle is about six weeks, but may vary from four to nine weeks depending largely upon the time of year as it affects existing temperatures. It seems probable that average temperatures in the low eighties (Fahrenheit) are optimum for most rapid development.

First eggs of the year are deposited by locally overwintering beetles during March, oviposition being continuous from March through August. The first egg deposition in peanut fields occurs around early June.

Some rootworm injury to peanut pods may be apparent by early July. Most of the damage was observed, however, to occur during August and September and maximum rootworm populations were observed during late August.

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ANISOPTERAN ODONATA OF THE SAVANNAH RIVER PLANT, SOUTH CAROLINA

By William H. Cross

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Introduction

During the period between September, 1951 and August, 1954 a collection of dragonflies was made in South Carolina on the United States Atomic Energy Commission Savannah River Plant (hereafter referred to as the S.R.P.). The collection was made as one of several studies of a broad ecological study program under a contract (No. AT(07-2)-10) between the University of Georgia and the Atomic Energy Commission. The S.R.P. includes portions of Aiken and Barnwell Counties as well as a narrow corridor in Allendale County. Only the first two counties, however, are represented in the collection.

The S.R.P. is situated on the upper edge of the Coastal Plain physiographic province very close to the Fall Line. According to physiolographic classification by Cooke (1936) the S.R.P. includes two distinct sub-regions. The lower of these, the Coastal Terraces sub-region, is composed of the Savannah River swamp and a band of paralleling dry land territory. It is characterized by level topography and an altitude of less than 200 feet. The upper sub-region which comprises the greater portion of the S.R.P. is a part of the Aiken Plateau with elevations greater than 200 feet except in the major stream valleys.

Although there are no large lakes present, other types of watery environments are well represented. The Savannah River bounds the southwest edge of the S.R.P. and is also the South Carolina-Georgia line. Its flood plain, which on the South Carolina side averages almost two miles in width, is largely flooded at times of high water and contains numerous water-filled pools and oxbows at times of low water. Five major stream systems of a clear water, sandy-bottomed type dissect the S.R.P. Stream swamps are not extensive. A number of artificial ponds were built by private landowners before the land was acquired by the government. Several of these were drained at various times during the study. Except for the above mentioned pools and oxbows of the river swamp, Carolina bays represent practically the only bodies of permanent static water of natural origin found on the S.R.P. These occur in various sizes but are mostly rather small.

Dragonflies have received relatively little study in South Carolina. Montgomery (1940) compiled "The Odonata of South Carolina" in which he summarized past literature and incorporated new records, listing 61 species of Anisoptera for the state. He records 10 species for Aiken County and 3 for Barnwell County.

The present collection was made with the purpose of sampling all of the available habitats. Little search for nymphs was attempted. Forty-eight species were found of which the following 15 are apparently new state records: *Gomphus ivae*, *Dromogomphus spoliatus*, *Gomphaeschna antilope*, *Basiaeschna janata*,

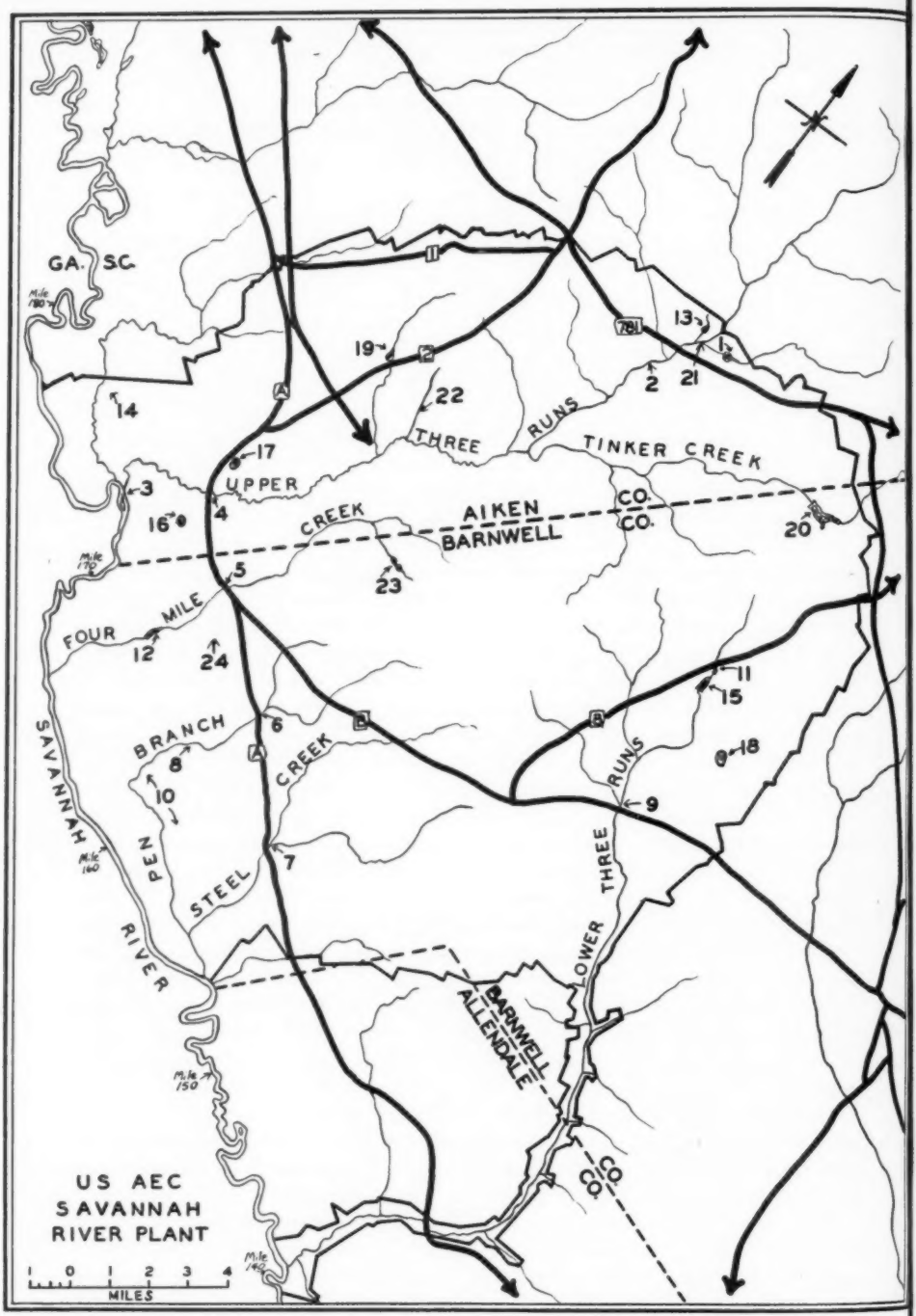

FIG. 1. The Savannah River Plant boundary is represented by the irregular line and the numbers 1–24 represent stations at which most of the dragonflies were collected.

Nasiaeschna pentacantha, *Macromia taeniolata*, *Neurocordulia* sp., *Epicordulia regina*, *Tetragoneuria spinosa*, *T. williamsoni*, *Helocordulia selysii*, *Somatochlora georgiana*, *Nannothemis bella*, *Celithemis verna*, and *Sympetrum vicinum*. All species of uncertain identity were very kindly checked by Dr. Minter J. Westfall, Jr. for which the writer is deeply appreciative.

A generalized map of the S.R.P. (Fig. 1) indicates the positions of 24 stations at which most of the specimens were collected. Artificial ponds are represented by stations 1, 11, 12, 13, 15, 19, 20, 23; Carolina bays, by stations 16, 17, 18; streams, by stations 2, 4, 5, 6, 7, 8, 9, 21, 22; river and stream mouth, by station 3; river swamp edge and oxbows, by stations 10, 14; and borrow pits, by station 24. It is obvious that the artificial pond stations also represent stream stations to some extent since they all occur in stream valleys. Several species were found at considerable distances from any bodies of water especially over abandoned fields of the S.R.P.

Fig. 2 includes species collected as adults on three or more dates and gives some indication of their seasonal occurrence. A bar extends from the date of earliest collection to the latest. Some species were found to occur as adults only during spring months and others only during fall months, whereas a few were found throughout most of the year.

ANNOTATED LIST OF SPECIES COLLECTED ON THE SAVANNAH RIVER PLANT

Tachopteryx thoreyi (Hagen). Stations 1, 2. 8 adults. April 23–June 30. Most often from light-colored tree trunks or from the walls of buildings where they often hung motionless.

Progomphus obscurus (Rambur). Stations 1, 21. 1 adult male. June 29. Nymphs rather common in sandy bottoms of streams.

Hagenius brevistylus Selys. Stations 1, 3, 4. 4 adults. June 30–October 7. From three points along Upper Three Runs, largest stream on the S.R.P. Pair taken in tandem on August 16.

Erpetogomphus designatus Hagen. Station 3. 3 adults. June 16–October 7. From the vicinity of Ellenton Landing on the Savannah River.

*Gomphus parvidens** Currie. Stations 1, 2. 11 adults. April 4–May 24. From two points near Upper Three Runs.

Gomphus ivae Williamson. Stations 8, 9, 10 adults. September 28–October 11. Rather easily netted as they few up and down the stream and perched on overhanging branches.

Gomphus laurae Williamson. Station 1. From two nymphs collected in a stream on May 6 a female emerged on May 20 and a male on May 28.

Gomphus plagiatus Selys. Station 3. 1 adult male. October 4. Taken while skimming over the stream with a number of others.

Gomphus lividus Selys. Stations 10, 22. 3 adults. April 3–12. From border of Savannah River swamp and along a small clear stream.

* Since this paper has gone to press the following note has become necessary: the specimens listed above as *Gomphus parvidens* Currie are currently being considered by Mr. George H. Beatty as a possible new species very closely allied to *G. parvidens*.

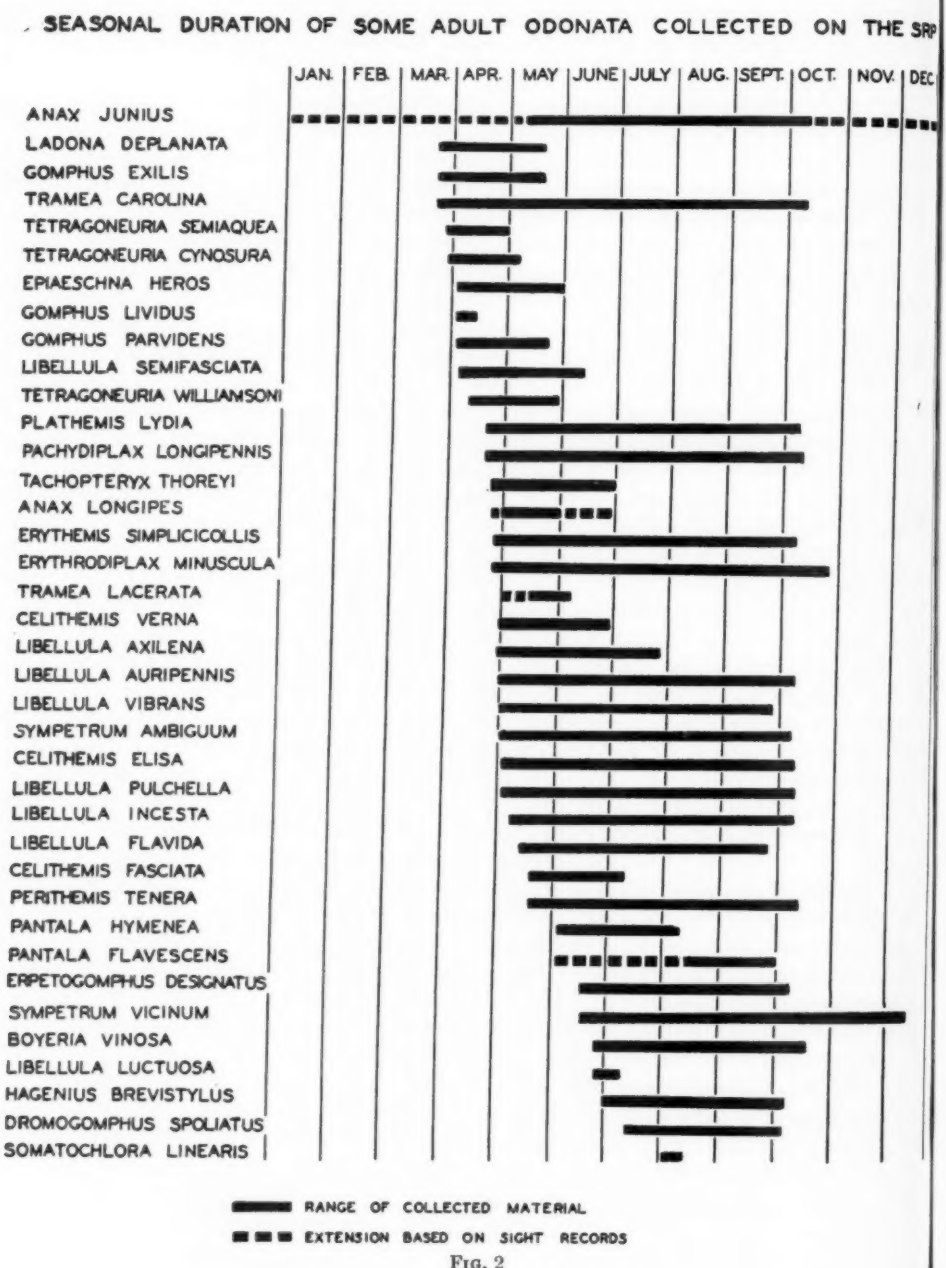
SEASONAL DURATION OF SOME ADULT ODONATA COLLECTED ON THE SRP
JAN.
FEB.
MAR.
APR.
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
ANAX JUNIUS
LADONA DEPLANATA
GOMPHUS EXILIS
TRAMEA CAROLINA
TETRAGONEURIA SEMIAQUEA
TETRAGONEURIA CYNOSURA
EPIAESCHNA HEROS
GOMPHUS LIVIDUS
GOMPHUS PARVIDENS
LIBELLULA SEMIFASCIATA
TETRAGONEURIA WILLIAMSONI
PLATHEMIS LYDIA
PACHYDIPLAX LONGIPENNIS
TACHOPTERYX THOREYI
ANAX LONGIPES
ERYTHEMIS SIMPLICICOLLIS
ERYTHRODIPLAX MINUSCULA
TRAMEA LACERATA
CELITHEMIS VERNA
LIBELLULA AXILENA
LIBELLULA AURIPENNIS
LIBELLULA VIBRANS
SYMPETRUM AMBIGUUM
CELITHEMIS ELISA
LIBELLULA PULCHELLA
LIBELLULA INCESTA
LIBELLULA FLAVIDA
CELITHEMIS FASCIATA
PERITHEMIS TENERA
PANTALA HYMENEA
PANTALA FLAVESCENS
ERPETOGOMPHUS DESIGNATUS
SYMPETRUM VICINUM
BOYERIA VINOSA
LIBELLULA LUCTUOSA
HAGENIUS BREVISTYLUS
DROMOGOMPHUS SPOLIATUS
SOMATOCHLORA LINEARIS
RANGE OF COLLECTED MATERIAL
EXTENSION BASED ON SIGHT RECORDS

FIG. 2

Gomphus exilis Selys. Stations 1, 2, 11, 12, 13. 17 adults. March 22–May 17. Apparently the most common gomphine on the S.R.P. during the spring months. Adults begin emerging from standing bodies of permanent water in the last part of March.

Dromogomphus spoliatus Hagen. Stations 1, 2, 14. 3 adults. July 13–October 3.

Dromogomphus spinosus Selys. Station 3. 1 adult male. October 4. From Ellenton Landing where this and other individuals were flying just above the water and alighting on small fishing boats. Collections of *Dromogomphus* suggest an association with the river for *D. spinosus* and an association with the small clear streams of the S.R.P. for *D. spoliatus*.

Gomphaeschna antilope Hagen. Station 1. 6 adults. May 21. These and several others were flying with a considerable number of *Epiaeschna heros* at places where winged termites were emerging. Emergence of termites from even a single rotten tree may thus present a golden opportunity for the capture of dragonflies by concentrating and holding in one place species which are otherwise seldom seen and difficult to capture.

Basiaeschna janata Say. Stations 10, 15. 11 adults. March 22–April 2. On the latter date common over fields bordering the Savannah River swamp.

Boyeria vinosa (Say). Stations 1, 2. 5 adult males. June 24–October 16. A crepuscular species which often becomes trapped in buildings about dusk.

Anax longipes Hagen. Stations 1, 13, 16, 17. 6 adult males. April 24–June 29 (including some sight records). From artificial ponds and Carolina bays. Appears to be less common than *A. junius* and is possibly somewhat more difficult to net.

Anax junius (Drury). Stations 1, 12, 17, 18. 5 adults. May 7–October 8. Also seen flying during several of the winter months, including December and February, over Carolina bays in both Aiken and Barnwell Counties. Apparently one of the few species which may overwinter as an adult in this area.

Epiaeschna heros (Fabricius). Stations 1, 10, 19. 10 adults. April 2–May 31. This aeschnine is one of the largest species found on the S.R.P. As with several others, it is at times also somewhat crepuscular in habit. On two occasions *E. heros* was seen in considerable numbers feeding on emerging winged termites.

Nasiaeschna pentacantha (Rambur). Station 14. 1 adult male. July 30. From an oxbow in the Savannah River swamp.

Macromia taeniolata Rambur. Stations 7, 14. 2 adults. July 9–30.

Macromia georgina Selys. Station 3. 1 adult male. October 4. From bank of Savannah River at Ellenton Landing.

Neurocordulia sp. Station 1. 1 adult female. July 18. Attracted by an artificial light to a screened porch at 10:45 p.m. This is the specimen mentioned by Westfall (1953). On December 8 a late instar nymph was collected in a small stream (also station 1) and on October 31, 1951, another late instar nymph was collected by Dr. D. C. Scott (Savannah River Studies, U. S. Public Health Service) at mile 171.52 on the Savannah River which is just above the Aiken-Barnwell County line.

Neurocordulia molesta Walsh. From September 4 to October 4, 1951, Dr. D. C. Scott collected over 60 nymphs of *N. molesta* in the Savannah River at five localities between mile 170.15 and mile 172 (just below the Aiken-Barnwell County line to just above the mouth of Upper Three Runs). The nymphs of this species mentioned by Westfall (1953) as coming from the Savannah River were collected in the same general area.

Epicordulia regina Selys. Station 1. 1 adult male. June 10. This individual was seen flying about with several others over an artificial pond and had to be captured from a boat since it approached shore with only the utmost caution.

Tetragoneuria semiaquea Burmeister. Stations 1, 13, 19. 40 adults. March 27–April 29. From three artificial ponds. A number of the adults emerged within screen traps placed in the shallow edge of one of these ponds so that adult and exuviae were recorded as the same individual. Some were observed while emerging to the adult, leaving the exuviae clinging a few inches above the water on a board, log, or blade of grass. Emergence occurred at least as late as April 13.

Tetragoneuria spinosa Hagen. Station 10. 10 adults. April 2–13. Rare in collections. From along the border of the Savannah River swamp. On the first date two pairs were taken while flying in tandem. Both males and females are somewhat larger than those of *T. cynosura*, a number of which were taken at the same time.

Tetragoneuria cynosura Say. Stations 1, 10, 12, 20. 50 adults. March 28–May 2. Probably the commonest *Tetragoneuria* on the S.R.P. Emergence of *T. cynosura* was noted on April 4 and 8 at an artificial pond (station 1). The series of specimens taken clearly demonstrates a progressive increase in color at the base of the hind wings from the typical *cynosura* type to the *simulans* type as these were separated by Muttkowski (1911). It is clear that this species, as well as the entire genus, needs considerable study.

Tetragoneuria williamsoni Muttkowski. Stations 1, 20. 32 adults (probably this species). April 11–May 27. From two artificial ponds. Emergence was noted April 11, 12, and 28.

Helocordulia selysii (Hagen). Station 2. 3 adult males. March 30–April 4. From along Upper Three Runs where they were often hovering just above the water as almost invisible objects, first in a sun fleck, then in the shade of an overhanging branch.

Somatochlora georgiana Walker. Station 1. 1 adult pair. July 23–30. Both from the deep shade of a grove of small water oaks along a small stream.

Somatochlora linearis (Hagen). Stations 4, 10, 14. 3 adults. August 4–10.

Nannothemis bella Uhler. Station 1. 1 adult male. May 17. From the grassy border of an artificial pond. *N. bella* and *Erythrodiplax minuscula* may very often be overlooked owing to their small size and their habit of making only short flights while hidden in tall grass.

Perithemis tenera (Say). Stations 1, 18, 19, 23. 11 adults. May 17–October 7. A small attractive species which frequents bodies of standing water. The males spend most of their time skimming a fraction of an inch above the water surface

and alighting upon low objects, while the females usually remain more inconspicuous in bordering vegetation.

Celithemis eponina (Drury). Stations 1 (sight records only), 16. Several sight records were made during June at an artificial pond and on July 29 a female was collected in a field near a Carolina bay.

Celithemis elisa (Hagen). Stations 1, 18, 19, 23, 24. 13 adults. May 2–October 7. From two artificial ponds, a large Carolina bay, and a small borrow pit.

Celithemis fasciata Kirby. Stations 1, 18. 21 adults. May 17–July 7. From artificial and natural ponds. Between May 21 and 31 a number of adults were taken with their exuviae in screen traps.

Celithemis amanda Hagen. Station 1. 1 adult male. July 7. From the border of an artificial pond.

Celithemis ornata (Rambur). Station 18. 3 adults. July 13–14. From a large Carolina bay.

Celithemis verna Pritchard. Stations 1, 17. 8 adults. May 17–June 29. From an artificial pond and a Carolina bay. One individual taken at night in a light trap. This species possesses almost no color at the bases of the hind wings and since it turns dark blue with age, it might easily pass for a large *Erythrodiplax minuscula*.

Erythrodiplax minuscula (Rambur). Stations 1, 3, 12, 17, 18, 19. 31 adults. April 29–October 25. A diminutive species which frequents grassy areas particularly about pond margins.

Ladona deplanata (Rambur). Stations 1, 11, 12, 20. 11 adults. March 22–May 17. A pond frequenting species which, together with *A. junius*, *G. exilis*, and species of *Tetragoneuria*, is seen on the wing after the first warm days of spring.

Libellula luctuosa Burmeister. Station 1. 4 adults. June 27–July 7. From the border of an artificial pond.

Libellula auripennis Burmeister. Stations 1, 15, 16, 17, 18, 23. 18 adults. May 1–October 8. Found on several artificial ponds and Carolina bays.

Libellula flavida Rambur. Stations 1, 5, 7. 7 adults. May 13–September 26.

Libellula semifasciata Burmeister. Stations 1, 3, 4, 10, 17, 18, 22. 18 adults. April 6–June 16. Found over fields well away from water as well as over artificial ponds and Carolina bays.

Libellula pulchella Drury. Stations 3, 17, 18. 12 adults. May 2–October 8. At artificial ponds, at Carolina bays, over fields, and along the Savannah River.

Libellula incesta Hagen. Stations 1, 2, 3, 4, 12, 14. 24 adults. May 7–October 7. From ponds and along streams. A teneral was collected on May 7 and an an emerging female on May 29.

Libellula vibrans Fabricius. Stations 4, 5, 6, 10, 14. 14 adults. May 2–September 27. From forest edges and along creek swamps.

Libellula axilena Westwood. Stations 1, 5, 17, 18, 23. 11 adults. May 1–July 28. Most often seen at swampy situations.

Plathemis lydia (Drury). Stations 1, 3, 6, 14, 23. 13 adults. April 19–October 7. Found at forest edge, at pond edge, and along streams.

Sympetrum ambiguum (Rambur). Stations 1, 3, 7, 12, 18. 20 adults. May 2–

October 5. Found at artificial ponds, at one Carolina bay, and along streams. On May 2 at small, shallow pools near the Savannah River (station 3) a number of tenerals were seen but no exuviae were found.

Sympetrum vicinum Hagen. Stations 11, 14, 18. 5 adults. June 16–December 13. On artificial ponds, in the river swamp, and on a Carolina bay. Apparently a very cold-hardy species, as the December 13 collection would seem to indicate. On the nights of November 19, 20, and 21 the average minimum temperature was about 16°F. and during the night of December 12 the minimum was 27°F. December 13 was a clear day with a high of 58°F. Emergence of these individuals after the November cold spell seems unlikely since all had the color of well aged individuals.

Pachydiplax longipennis (Burmeister). Stations 1, 3, 7, 12, 13, 16, 17, 18. 25 adults. April 19–October 8. One of the most common dragonflies on the S.R.P. A female was taken during the night of July 29 in a light trap.

Erythemis simplicicollis (Say). Stations 1, 16, 17, 18. 13 adults. April 29–October 6. From artificial ponds, Carolina bays, and stream swamps. Two males were taken during the night in light trap samples, one on July 15 and one on July 29.

Pantala hymenea (Say). Stations 6, 16. 3 adults. June 2–August 6.

Pantala flavescens (Fabricius). Stations 5, 16. 3 adults. June 2–October 1 (including sight records). The scarcity of specimens of *Pantala* obtained by no means indicates low population. Actually they are of common occurrence especially during late summer. Both species are often seen flying over temporary rain pools particularly along dirt roads.

Tramea lacerata Hagen. Stations 1, 16. 6 adults. May 1–June 4 (including sight records). Over fields, on two artificial ponds, and on one Carolina bay.

Tramea carolina Linnaeus. Stations 1, 10, 11, 16, 17, 18. 34 adults. March 22–October 6. One of the most conspicuous and wide ranging dragonflies on the S.R.P. As in the case of *Anax junius*, overwintering possibly occurs.

SPECIES COLLECTED NEAR THE SAVANNAH RIVER PLANT

Gomphus vastus Walsh. 1 adult female. May 22. From the bank of the Savannah River at Shell Bluff Landing, Burke County, Georgia. This is immediately above the S.R.P.

Cordulegaster diastatops Selys. 2 adult males. April 20. Flying along a small stream which flows down the bluff above the Savannah River flood plain at a point about one mile south of Tahoma, Richmond County, Georgia. This is about seven miles from the nearest S.R.P. boundary.

Neurocordulia virginiensis Davis. On July 27, 1952, a late instar nymph was collected by Dr. D. C. Scott (Savannah River Studies, U. S. Public Health Service) at mile 201.55 on the Savannah River about 10 miles northwest of the S.R.P.

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DISTRIBUTIONAL RECORDS FOR SOME SCARCE PHALANGIDS IN THE SOUTHERN APPALACHIANS

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In the process of collecting for diplopods I often encounter other small arthropods about which little is known. One such group of animals is the Order Phalangida, represented on one hand by the abundant and conspicuous "Daddy long-legs" and on the other by tiny humus-dwelling members of the families Ischyropsalidae and Phalangodidae.

Little has been written on the phalangid fauna of the Southern Appalachians since the paper of Crosby and Bishop (Jour. Elisha Mitchell Sci. Soc. **40:** 8–26) in 1924. These workers recorded and described several species of the small forms, chiefly from Grandfather Mountain, Mount Mitchell and Mount Pisgah. In recent years I have been fortunate in being able to collect material of various small phalangids, some of which provide distributional records of sufficient local interest to warrant their publication. It is hoped that other zoologists collecting in the region will be stimulated to pick up these interesting creatures, of which numerous undescribed species doubtless remain to be discovered in the Appalachian region.

In addition to specimens personally collected, I include reference to several collections at Cornell University.

Family ISCHYROPSALIDAE

Sabacon cavicolens Packard

Crosby and Bishop recorded this species from Grandfather Mountain and Blowing Rock, N. C. Originally described from Kentucky, it has since been recorded from most of the New England states, from Wisconsin, and from the Pacific Northwest. The following collections represent new state records, and carry the known range somewhat farther to the southeast.

VIRGINIA. Alleghany Co.: 3 mi. n.w. Clifton Forge, cold moist cove forest, Sept. 10, 1948. Giles Co.: Mountain Lake, 3800 ft., Sept. 27, 1950. TENNESSEE. Sevier County: Laurel Creek, Oct. 8, 1926. (Cornell collection). SOUTH CAROLINA. Greenville County: Greenville, Oct. 4, 1930, Norman W. Davis (Cornell collection).

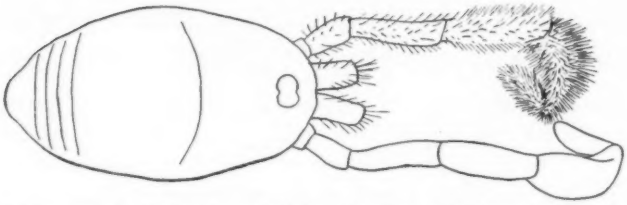

FIG. 1. *Sabacon mitchelli* Crosby and Bishop. Outline of body and palps, dorsal view. Legs and color pattern omitted, as well as the setae from the right palpus. Female specimen from Highlands, North Carolina.

Although most workers have come to regard this species as the same as *S. crassipalpe* Koch from Siberia, I follow the precedent of Crosby and Bishop in keeping the two separate until adequate comparisons of specimens is made. The genus is badly in need of careful study.

Sabacon mitchelli Crosby and Bishop. Fig. 1.

This species has not been taken, apparently, since the type was found on Mount Mitchell at an elevation of 6000 feet. On June 2, 1954, I obtained another female, apparently of this species, in a rhododendron thicket along the Cullasaja River about 4 miles west of Highlands, North Carolina.

S. mitchelli appears to be a smaller species than its relative *cavicolens*, and to be somewhat less spinose. The specimen at hand is not in full color but otherwise agrees well with the original description and differs correspondingly from specimens of *cavicolens*.

An outline drawing of this example is presented to show the shape of the body and of the palpi. Members of the genus are immediately recognizable by the enlarged and densely setose terminal joints, giving the appendages the appearance of bottle brushes.

Family PHALANGIIDAE

Caddo agilis Banks.

Originally described from Long Island, this species has been found at other New York localities and at Washington, D. C. In 1911 (Proc. Acad. Nat. Sci. Philadelphia, p. 456) Banks reported specimens collected at Swannanoa, Buncombe County, North Carolina, a record which seems to have been generally overlooked. Caddo appears to be quite scarce, as evidenced by the few known localities and by the fact that three specimens are the most I have ever found at one time.

NORTH CAROLINA. Macon Co.: 4 mi. w. Highlands, along the Cullasaja River, June 2, 1953 (one specimen under a dry stump in a rhododendron thicket). VIRGINIA. Alleghany Co.: Warm Spring Mountain, 3600 ft., 6 mi. w. of Clifton Forge, May 15, 1954 (3 specimens under logs on a dry shale slope). Craig Co.: Potts Mountain, 3200 ft., 2 mi. e. Paint Bank, July 17, 1951 (one specimen in moss, moist shady woods). Montgomery Co.: 1 mi. e. Blacksburg, 2000 ft., July 10, 1951 (one specimen on a dry exposed limestone cliff, collected at night).

The few observations on habitat indicate a considerable tolerance of, or even preference for, dry situations. The yellow pigmentation of living specimens is quite pronounced, and, with the peculiar enlargement of the eye tubercule, makes field recognition of *Caddo* easy. Despite the small size and shortened legs, the species has preserved the curious gait of the larger, long-legged harvestmen.

THE AMPHIBIANS OF NEW HANOVER COUNTY, NORTH CAROLINA

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This report is based upon the observations and collections of amphibians made over a period of fourteen years in New Hanover County, where little previous work has been done on the reptiles and amphibians.

Myers (1924) did no actual collecting in this county, basing his report on specimens sent to him by Mrs. J. H. Cummings of Wilmington, who was an assiduous collector but published no papers. He listed seven species of salamanders, and fourteen species of frogs and toads in his paper. C. S. Brimley (1941) brought the records for North Carolina up to date and reported three additional species of salamanders. He also listed fourteen species of frogs and toads, and, giving the range of *Bufo fowleri* and *Acris crepitans* as "probably state-wide," included these two species by inference but gave no definite records for the county. This report gives the status and distribution of these forms within the county and adds four species of salamanders and five species of frogs and toads not heretofore known from New Hanover County.

New Hanover County occupies a triangular area, formed by the junction of the Atlantic Ocean and the Cape Fear-North East River, in the lower coastal plain of southeastern North Carolina. At the tip of the peninsula and for some miles inland the terrain consists of sand dunes with a dominant vegetational cover of turkey oak (*Quercus catesbaei*), margarete oak (*Q. margaretta*), running oak (*Q. cinerea*) and black jack oak (*Q. marilandica*). With the exception of a few characteristic herbs and clumps of wire grass (*Aristida stricta*) there is no ground cover and amphibians are generally absent, except where upland bogs have formed in low places within the dunes. A narrow fringe along the coast is maritime climax forest with the live oak (*Q. virginiana*) and the following evergreen shrubs as the dominant plants: dahoon holly (*Ilex cassine*) and smilax (*Smilax laurifolia*). The Cape Fear-North East River is bordered by swamps of two types. Where flooding is continuous, bald cypress (*Taxodium distichum*) dominates, but in areas subject only to seasonal flooding, gum swamps are found with southern black gum (*Nyssa biflora*), tupelo gum (*Nyssa aquatica*) and water ash (*Fraxinus caroliniana*)

as dominants. Cypress-bordered Greenfield Lake, within the Wilmington city limits, is the only large lake in the county.

The remainder of the county consists of cultivated land and pine savanna. The higher portions of these savannas are open stands of long-leaf pine (*Pinus palustris*) and loblolly pine (*P. taeda*) with a ground cover of wire grass, while the lower undrained areas form upland bogs or pocosins. Here the pond pine (*P. serotina*) and evergreen shrubs such as leatherwood (*Cyrilla racemiflora*), red bay (*Persea borbonia*), swamp-bay magnolia (*Magnolia virginiana*) and loblolly bay (*Gordonia lasianthus*), thickly overgrown with smilax, are the most conspicuous plants.

These savannas owe their origin and maintenance to their resistance to fire. If protected from fire they would be replaced by oak-hickory forest, but no extensive areas exist where fire has been excluded for more than a relatively few years (*Oosting*, 1948). It is because of this, I feel certain, that amphibians of all types are rare in the savanna areas, except where there are small ponds, and even here, if a fire is severe enough, all forms of life disappear. Since in New Hanover County these savannas are burned almost yearly, no large populations can exist. The only salamander that I have found in the savannas is *Stereochilus marginatus*, which is more aquatic and mud-burrowing than other forms, and can undoubtedly survive most fires.

A. H. Wright in his "Life-Histories of the Frogs of Okefinokee Swamp, Georgia" considers Wilmington, North Carolina, to be within his "Sabalian" zone, and under his account of *Pseudacris ocularis* he states: "Wright (1926, p. 82) gives *Pseudacris ocularis* as a Sabalian species from South Carolina to Florida. Inasmuch as Wilmington, N. C. is Sabalian country it is not surprising to find it recorded there". Wright's Sabalian zone is equivalent to the swampy coastal plain zone of Van Dersal and Mulford (Wright 1949, p. 8) which coincides with the range of the Sabal Palmetto.

Under his general discussion of the Okefinokee Salientia, Wright has divided the frogs of the swamp into two groups. Eleven species are considered Sabalian forms and nine species are considered Austral. However, an attempt by the present author to classify the species of frogs in the Wilmington area into two distinct groups as Wright has done was unsuccessful. The ranges of the frogs and toads in Wright's "Handbook of Frogs and Toads", and of the salamanders in Bishop's "Handbook of the Salamanders", were checked against Merriam's Life Zones, as given in Wright (1949, p. 6). During this study, the probable reason for the presence of such Upper Austral forms as *Plethodon c. cinereus*, *Pseudotriton m. montanus*, *Pseudotriton r. ruber*, *Bufo fowleri* and *Pseudacris nigrita feriarum* in the lower coastal plain was brought into focus.

It seems plausible to assume that the southern movement of the ice sheets during the glacial periods caused a lowering of the temperature in the southern part of the American continent. This decrease in temperature would cause the life zones as we know them to shift southward also and during this period the coastal plain was probably more suitable for Upper Austral forms than it was for Lower Austral associations. During this time Upper Austral forms were probably widespread throughout this area.

With the final withdrawal of the glaciers, the limits of the Upper Austral Zone moved inland from the coast, and northward, and the life zones became stabilized as we know them today. As the zones moved, animal life associated with each zone moved with it or died out, except where conditions were suitable for continued existence. The upper Austral forms named above seem to be relict populations dating from that time, *Bufo fowleri* and *Pseudacris nigrita feriarum* surviving because of tolerance to changing conditions, and the salamanders because they had found a suitable habitat.

This theory is borne out by the fact that those salamanders of the coastal plain which are considered Upper Austral forms are invariably found in habitat niches which strongly resemble Piedmont forest and, as such, differ markedly from typical coastal plain forest types. The discontinuous distribution of *Plethodon glutinosus* in the coastal plain can also be explained in this manner, since this species too is found only in areas which appear Upper Austral in aspect.

The writer is indebted to Mr. C. H. McAllister of Wilmington, N. C. for many specimens and constant assistance on numerous field trips, and to Dr. Theodore H. Eaton of East Carolina College, Greenville, N. C., for his advice and criticism in connection with this paper.

Scaphiopus holbrookii holbrookii Harlan. Eastern Spadefoot Toad

Neither Myers (1924) nor Brimley (1941) recorded *Scaphiopus h. holbrookii* from New Hanover County. This species is abundant, however, and during its breeding congress can be found in great numbers. At other times they can often be found hopping about at night during heavy rains. I have records of breeding activities as early as April 18, 1941 and as late as November 3, 1948. Brandt (1936) states that he has never found more than one breeding congress in a season in Beaufort County and Brimley (1941) makes the same statement for Raleigh, but for 1949, a year of exceptionally heavy and prolonged rains, I have records of three breeding periods for this form in this county. The three dates were May 1, June 12, and August 21, on the last of which the largest congress of all took place, spadefoots appearing simultaneously in a number of localities. At one such area, a flooded field, I took seven specimens, including two mated pairs. This is the only year in the period during which observations were made by the present author in which more than one breeding congress was observed by the author to take place, but Wright (1932) in his account of this species gives a record of four separate and distinct appearances of the Spadefoot at Cambridge, Massachusetts in one season, each during or immediately following a very heavy fall of rain.

Bufo woodhousei fowleri Hinckley. Fowler's Toad

Myers (1924) did not give any record of this form for the county, while Brimley (1941) gave its range as "nearly state-wide, overlapping that of the Southern Toad, *Bufo terrestris*, in the coastal plain". These two species are equally abundant in New Hanover County. Brandt (1936) states that *Bufo fowleri* is abundant in Beaufort County but concentrates in different foci from those occupied by *Bufo terrestris*. This seems to be true for this area also, except during the breed-

ing season, when both forms may be found in the same breeding areas. *Bufo fowleri* seems to prefer the vicinity of water in its habitat selection here, while *Bufo terrestris* can be found in much drier locations. The earliest breeding record for this area is March 13, 1950 and the latest date is May 31, 1948.

Bufo terrestris terrestris Bonnaterre. Southern Toad

Myers (1924) and Brimley (1941) both gave records for this toad in New Hanover County. It is a common species. The earliest spring appearance on record is March 23 and breeding occurs sporadically as late as June 26.

Bufo quercicus Holbrook. Oak Toad

Myers (1924) recorded two specimens of this toad from the county and Brimley (1941) also gave New Hanover in its range. It is a very common pine-woods form and its high-pitched call can be heard during all the summer months in rainy seasons. The earliest breeding record I have for this locality is May 12 1948, indicating a later breeding period than our other two species of *Bufo*.

Acris gryllus gryllus Le Conte. Coast Marsh Cricket Frog

This small frog has been reported by Myers (1924) from New Hanover. It is our most common species and can be taken during any month of the year, except during very cold weather. The earliest breeding record I have for this species is February 5, 1949.

Pseudacris nigrita nigrita Le Conte. Southern Chorus Frog

This frog has not been reported from New Hanover County, although it has been taken in other coastal plain localities (Brandt, 1936). It is widely distributed throughout the county, especially in road-side ditches overgrown with vegetation. This species and the next call sporadically during warm days all through the winter, but February marks the beginning of the breeding season for both species.

Pseudacris nigrita feriarum Baird. Eastern Chorus Frog

The Eastern Chorus Frog is another species which has not heretofore been known from New Hanover County. It is much rarer than *Pseudacris n. nigrita* and is not found in the same kind of habitat. *Pseudacris n. feriarum* is found in the shallow ponds of the pine savannas and is one of the most difficult of our frogs to secure. It is partial to flooded thickets growing at the edges of and scattered at random throughout these ponds. It is rarely seen out of the water and seems to be far more aquatic than *Pseudacris n. nigrita*.

Pseudacris ornata Holbrook. Ornate Chorus Frog

Heretofore known only from the vicinity of Washington, in Beaufort County (Brandt, 1936), this chorus frog has been taken in New Hanover County on only one occasion, when a small adult was collected on April 25, 1953 by C. H. McAllister and the author at the edge of a small pond in the pine savanna south of Wilmington. This specimen was not calling but was seen hopping through the

short grass on the edge of the pond. During the early afternoon of December 23, 1953, a warm sunny day, a number of these frogs were heard calling in a small pond near Castle Hayne, but, owing to the density of the vegetation in and around the pond, it was impossible to secure specimens.

Hyla ocularis Bosc and Daudin. Least Tree Frog

Myers (1924) referred to one specimen of this frog mentioned by Noble from Wilmington as the first record of this species from North Carolina. Brimley (1941) gave New Hanover in its range, but stated he had not heard its call. Beginning in April and continuing throughout the summer months the quivering insect-like trill of these frogs can be heard in any dense thicket. They are very partial to vine-covered clumps of low vegetation and are usually found perched among the vines, being found in the water only during the breeding period. Some individuals have been heard as early as February 3.

Hyla squirella Sonnini and Latreille. Southern Tree Frog

This frog has been reported previously from New Hanover by Myers (1924) and Brimley (1941). It is a very common species particularly among the cypress bordering Greenfield Lake, where it is found among the vegetation growing around the bases of the trees.

Hyla cinerea cinerea Schneider. Green Tree Frog

Brimley (1941) and Myers (1924) both reported this frog from New Hanover. Two specimens received by Myers completely lacked the yellow stripe characteristic of the species, and this color phase is not uncommon among this species here. *Hyla cinerea* is more often found among the large leaves of the waterlilies and other water plants such as cat-tails than it is on trees, and it is abundant in Greenfield Lake. This species and the preceding begin their breeding activities in April.

Hyla femoralis Sonnini and Latreille. Pine Woods Tree Frog

Myers (1924) recorded one specimen of this frog from New Hanover and Brimley (1941) gave this county as part of its range. It is very common in the pine-wood areas of the county and has been heard calling as early as April 26.

Hyla versicolor versicolor Le Conte. Gray Tree Frog

Myers (1924) and Brimley (1941) recorded this species from New Hanover. It is not as common as the other tree frogs in this area. It has been heard calling as early as February 10, but reaches the peak of its breeding activity in May.

Hyla crucifer Weid. Spring Peeper

Myers (1924) did not record this species from New Hanover and Brimley (1941) stated that he had no records for this part of the state. However, it is common throughout the whole southeastern section of the state and is quite common in New Hanover. It begins breeding as early as February 10 and has

finished its breeding activities and returned to the woods before most of the other forms begin, as do those of Beaufort County (Brandt, 1936).

Hyla gratiosa Le Conte. Barking Tree Frog

The first specimen of this large tree frog to be taken in New Hanover County was found at Masonboro Sound on August 21, 1951 by C. H. McAllister. Although an extensive search was made for this species during the next breeding season, it was not until May 1, 1953, that they were again found, when a large chorus of these frogs was heard in an inaccessible swamp at the head of Whiskey Creek on Masonboro Sound. Others were heard calling at a small grassy pond near Winter Park the same evening, and one specimen was secured. On May 6, 1953, five specimens of *Hyla gratiosa* were secured from Silver Lake near Carolina Beach, including one mated pair. Since that time, they have been found in numerous other localities in New Hanover County. (Funderburg, 1953.)

Rana pipiens sphenocephala Cope. Southern Leopard Frog

Myers (1924) recorded three specimens of this frog from Wilmington and Brimley (1941) included New Hanover in its known range. It is very common wherever there is water. I have taken numerous specimens which were typical *sphenocephala* in appearance but lacked the white spot at the center of the tympanum, supposedly characteristic of this type. It begins breeding here in early February.

Rana palustris Le Conte. Pickerel Frog

Myers (1924) and Brimley (1941) recorded this frog from New Hanover, but it is very rare here. I have never found it around ponds and lakes but always along the bank of some creek deep in the swamp. Smith Creek, near Wilmington is the only locality in the county where it could be considered fairly common.

Rana clamitans Latreille. Green Frog

Myers (1924) recorded this species from Wilmington and Brimley (1921) gave its distribution as "state-wide". It is fairly common in this area and begins breeding in March.

Rana virgatipes Cope. Carpenter Frog

Myers (1924) recorded two specimens of this frog from Wilmington and Brimley gave New Hanover in its range in North Carolina. It is a rare frog in this area and of very local distribution. It is most often heard in small pine savanna ponds, but there is one small sphagnum-choked branch of Greenfield Lake where it is fairly common. It breeds in February and March.

Rana catesbeiana Shaw. Bullfrog

Myers (1924) recorded one specimen of this frog from Wilmington and Brimley (1941) gave its distribution as "state-wide". It is very common in this area

occurring along all water courses and in all ponds. It has been heard calling as early as February 10 but begins its breeding activities in April.

Microhyla c. carolinensis Holbrook. Narrow-mouthed Toad

Myers (1924) and Brimley (1941) both recorded this species from New Hanover. It is very common and can be found easily by turning over rocks and old boards in suitable locations. The earliest breeding record I have is March 30, but it reaches the height of its breeding activity in May and June and continues to breed sporadically well into September.

Necturus punctatus Gibbes. Dwarf Water Dog

Necturus punctatus is the only form of *Necturus* known from New Hanover and until 1923 two specimens in the National Museum from this county were the only *punctatus* known from North Carolina. (Brimley 1941.) Myers listed this species in 1924 as having been reported to him from Wilmington but had received no specimens. Since 1924 it has been found in numerous other localities in the state. It is not uncommon in the small ditches and backwaters leading from the larger watercourses. It prefers a muddy pottom with masses of vegetation or thick clumps of decaying leaves in which it can take cover. It also prefers running water and I have never taken it in lakes or ponds where the water is still.

Siren lacertina Linnaeus. Great Siren

This species and the next are both found in Greenfield Lake, within the Wilmington city limits, but the *Siren* is less common. One specimen taken in this lake on July 29, 1952 measured twenty-six inches in length with a circumference of 5½ inches. It is considered to be poisonous by the fishermen of this area. The *Siren* is more nocturnal in habit than *Amphiuma* and has been found in hollow logs and drainage pipes as well as burrowed in the mud, its favorite hiding place.

Amphiuma means means Garden. Two-toed Amphiuma

The "Lampus Eel" is a very common inhabitant of Greenfield Lake and other water-courses and is often taken by fishermen during daylight hours. It is more often seen feeding in shallow water at night, however, and is often seen in water so shallow that the upper half of the animal is uncovered. When the lake warms up during the summer these amphibians come to the surface often, expelling air in a sibilant hiss. I have taken specimens of this form from the stomachs of *Farancia abacura* and *Abastor erythrogrammus*, both forms probably taking the amphiuma as their main food item. I have once taken it from the stomach of *Natrix erythrogastra*. Although this form is carnivorous I have often watched an amphiuma feeding through a group of breeding frogs and toads but have never seen it eat one, and these anurans seemingly have no fear of the amphiuma. Brimley (1941) recorded this species and the preceding one from New Hanover County.

Diemictylus viridescens dorsalis Harlan. Broken-striped Newt

Brimley (1941) recorded this newt from Wilmington and stated "a land form is known to occur at least in the vicinity of Wilmington". Myers (1924) considered this form to be merely a local race rather than a subspecies of *Diemictylus viridescens*.

Brimley called this the "typical" coastal plain form and had taken intergrades between this form and *viridescens* from counties further inland. I have taken numerous specimens of this newt all over the county, including land and water forms and larvae, and all, without exception, have shown the broken red line edged with black characteristic of this species. It is more common in small ponds and lakes than it is in rivers and streams, being found only in the backwaters of these larger streams. The land form is rare and seems to be found only during extreme dry periods and these specimens are always small. The water form may be found at all times of the year.

Ambystoma opacum Gravenhorst. Marbled Salamander

This salamander has not been heretofore recorded from this county and I have taken it only once. It is probably the rarest of the salamanders that occur here, although it is common in other localities of the coastal plain. I found an adult male under an old log near Corbett's Pond on Middle Sound on May 7, 1947, but an extensive search at that time and on subsequent trips failed to reveal further salamanders of this species.

Ambystoma mabeei Bishop. Mabee's Salamander

This species has been taken near Wilmington by Dr. A. H. Wright of Cornell University and it has been found in the lower Cape Fear Basin also (Brimley, 1941).

Manculus quadridigitatus Holbrook. Dwarf Four-toed Salamander

One of these salamanders, the first for this county, was found under a piece of rusted tin at the edge of a small stream leading into Greenfield Lake on May 3, 1946. It seems to be a rare form in the state although it may be found commonly in a few scattered localities, since Brimley (1941) states that at Raleigh: "The adults seem to be rather gregarious in the breeding season, twenty-five or more often being found in the same breeding place". It is not uncommon in Pitt County, another coastal plain locality (Eaton, 1953).

Pseudotriton montanus montanus Baird. Eastern Mud Salamander

Myers (1924) records two specimens of this salamander from Wilmington, and Brimley (1941) includes New Hanover in its range, put Bishop (1947) does not include the coastal plain in the distribution of this form or the next one. It is the more common red salamander in this county and is most often found while digging for fish bait or in ditch digging. In this area it seems to be almost entirely a subterranean form and is seldom found under old logs or debris as it is in other localities.

Pseudotriton ruber ruber Sonnini. Northern Red Salamander

This form of the red salamander has been taken only twice in New Hanover County, once near Wrightsboro in 1947 and once on Masonboro Sound in 1949. Both specimens were found under old logs and were very vividly colored. It has not been reported from this county previously although Brimley (1941) gives its range in North Carolina as "the whole state east of the mountains".

Stereochilus marginatus Hallowell. Margined Salamander

This salamander has been collected at Wilmington by Brimley (Bishop, 1947) and three specimens were reported by Myers (1924). The adult of this salamander is very elusive and hard to find but the larvae may be taken in small ponds in the pine savannas where the water is very dark and acid. Five larvae taken from one of these ponds near Winter Park on April 25, 1953 are all about one inch in length, with a row of light spots, rather than lines, down the sides, but the heads are depressed as in the adult. Brimley (1941) stated that the larvae apparently transform in May and are then 2½ to 3½ inches in length, but specimens taken in the latter part of May in these ponds were still under two inches in length and show no signs of transformation. Bishop (1947) stated that the older larvae resemble the adults in general form and color.

Desmognathus fuscus auriculatus Holbrook. Coastal Plain Dusky Salamander

Myers (1924) records only one specimen of this salamander from Wilmington, but actually it is very common in the leaf mold and under rotten logs in swampy sections throughout the county. During dry seasons it is definitely inclined toward burrowing deeply into the swamp muck and is often turned up by bait diggers as far down as two feet. It is a very quick and agile salamander and often dives down crayfish holes when pursued.

Plethodon cinereus cinereus Green. Red-backed Salamander

The Red-backed Salamander is rare in North Carolina outside the mountains and has been found only in two coastal plain localities heretofore. Brimley stated in 1941 that he had found specimens in 1921 in Wake County and that two had been taken in Pitt County in 1902. Since that time four more specimens have been found in Pitt County (Easton, 1953). On July 1, 1953, I was fortunate enough to add another locality in the coastal plain to this list, when I found three of these salamanders under rotten logs at the edge of the Mott Creek swamp on the River Road about four miles below Wilmington. Two of these specimens are the red-backed phase while the other is lead colored on the back.

Plethodon glutinosus glutinosus Green. Slimy Salamander

This salamander, so common in other parts of the state, is rare in New Hanover County. It is usually found in the moist wood of decayed logs on well-drained slopes, very seldom being found under logs as it is in other localities.

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THE STATUS OF THE TURTLE *KINOSTERNON BAURI PALMARUM* STEJNEGER WITH NOTES ON VARIATION IN THE SPECIES

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In 1891 Garman described a new species of turtle, *Kinosternon bauri*, from specimens collected at Key West, Monroe County, Florida. Later Stejneger (1925: 463) described *K. b. palmarum* from Royal Palm Hammock (now known as Paradise Key), Dade County, Florida. Although both subspecies have generally been recognized, the ranges of the two forms have been variously and puzzlingly delimited by Carr (1940, 1952) and Schmidt (1953). Pope (1939) did not recognize the subspecies *K. b. palmarum*. Both Wright (1935) and Carr (1952) have discussed the occurrence of *palmarum*-like individuals outside the "range" of that form. With the recent acquisition of considerable material from southern Florida and the keys, we feel that sufficient information is available to clarify the geographic variation within this species.

We have examined 220 specimens of *K. bauri* from localities from Key West to Alachua County, Florida. Of the characters used by Stejneger for *K. b. palmarum*, we have studied most intensively the pattern and pigmentation of the

head skin and of the carapace laminae. The other characters used by Stejneger were investigated casually. In addition, the pattern of the mandibular beak and proportions between four shell measurements were studied. Shell measurements follow Carr (1952).

Pattern and pigmentation of the carapace laminae and of the head skin were studied by selecting individual specimens as norms (Table I and II), and comparing others to them. In this way we hoped to obtain an objective evaluation of the variation in these characters. The variation is shown in Map 1. The specimens were grouped into twelve geographic areas, which were then combined into four inclusive regions (Map 1). For these areas, it can be seen that in none is only one type of carapace present; in all at least two types are present, and in those with larger samples, there are three or more. In the area around the type locality of *K. b. palmarum*, there is a greater proportion of individuals with lightly pigmented carapaces, though these include only about half the sample, the others having carapaces so corroded as to be indeterminable. The holotype (USNM *61065*) is an individual with extremely translucent carapace laminae; the bony sutures are visible over the entire carapace. Specimens of this extreme are generally absent in the northern part of the range though turtles from the northern areas are by no means all dark. There seems to be a gradual increase in the percentage of lightly pigmented individuals from north to south on the mainland. Almost all the adult specimens from the Lower Keys have the carapace very dark and the three dorsal light stripes almost obliterated by encroachment of dark pigment.

TABLE I

Standard specimens used for comparisons of carapace laminae

Museum Number	Sex	Locality	Description
DBUF 2889	♀	5 mi. s. Paradise Key, Dade County	Pigment of carapace laminae much reduced; bone sutures quite apparent through clear laminae; dorsal light lines obscure
UMMZ 105931	♀	20.6 mi. w. Miami, Dade County	Pigment of carapace laminae reduced, most noticeable laterally; bone sutures not themselves apparent, but their position indicated by reduction of pigment in laminae in region immediately over them; dorsal light lines obscure
DBUF 1305	♀	Dade County	Darker than UMMZ 105931; carapace laminae lightened both laterally and above the bone sutures, but three dorsal light lines apparent
UMMZ 105927	♀	9.2 mi. n.e. Indian Prairie Canal, Glades County	Dark, with no lightening of carapace laminae; three dorsal light lines boldly apparent and of moderate width
UMMZ 104010	♀	Big Pine Key, Monroe County	Extremely dark; three dorsal light lines almost obliterated by encroachment of dark pigment in carapace laminae

TABLE II

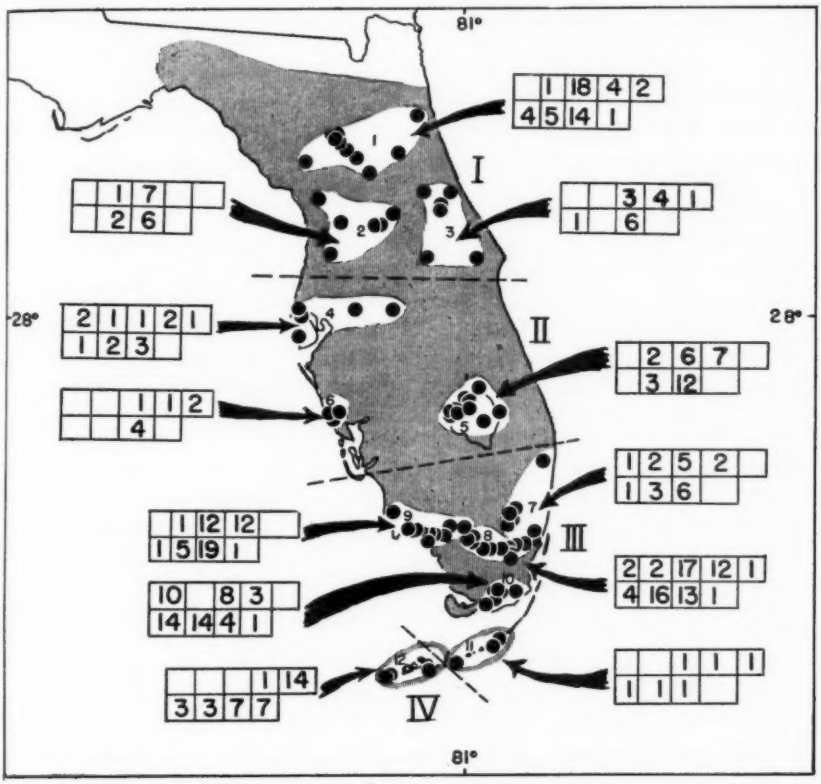
Standard specimens used for comparisons of head skin

Museum Number	Sex	Locality	Description
UMMZ 105955	♀	6 mi. e. Royal Palm Hammock, Collier County	Head skin gray, variously mottled; whitish or yellowish stripe arising at tip of snout and extending along neck, and similar line, beginning at angle of jaw, almost completely obliterated by added light mottling.
UMMZ 105960	♀	1 mi. n. Dade County line, Broward County	Head skin blackish; canthal and angular lines moderately well defined; some whitish stippling or mottling between lines and on lateral portions of neck
UMMZ 105927	♀	9.2 mi. n.e. Indian Prairie Canal, Glades County	Head skin black; canthal and angular lines sharply distinct from background; no mottling
UMMZ 104010	♀	Big Pine Key, Monroe County	Head skin black and unmottled; canthal and angular lines nearly obliterated by black pigment so that head appears almost entirely black

A geographic trend in head pattern is less obvious. In only one area is there a single type of head pattern, and in areas with larger samples there are at least two, and generally three or four. Less than half the specimens from near Paradise Key have mottled heads and indistinct head stripes, and this pattern is found wherever large samples are available, including the Lower Keys.

The variation in the pattern of the mandibular beak consists of differences in the amount of anteroposterior streaking on a usually dark background. Specimens were considered to have an unstreaked, a slightly streaked, or a heavily streaked lower jaw. A few unusual individuals were designated "spotted" or "light". Of twenty-three adult specimens from the Lower Keys, fourteen have the mandibular beak unstreaked, and nine are considered slightly streaked. Of 189 specimens from the Upper Keys and mainland, all are considered heavily streaked with the exception of two unstreaked, four slightly streaked, three light, and one spotted individual. No trend is apparent among the mainland or Upper Keys specimens. The specimens from the Lower Keys have consistently darker and less streaked mandibular beaks than those from any of the other populations, in which the preponderance of individuals with heavily streaked beaks is marked.

Three proportions were computed from shell measurements: width of bridge over carapace length ratio, anterior lobe of plastron over width of bridge ratio, and carapace width over anterior lobe of plastron ratio. No specimen under 55 mm. in carapace length was used for these ratios. Sexual dimorphism seems to be present in some populations for each of the ratios. The data, presented in Table III, are therefore grouped by sex for each area. In the width of bridge over carapace length ratio for the females there appears to be a cline running north-south from an average ratio of 2.2 in Alachua County through 2.1 near Lake Okeecho-



MAP 1. The range of *Kinosternon bauri* is shaded. The numbered unshaded areas with black locality dots represent the twelve areas from which specimens were examined. The dashed lines separate the range into four regions designated by the Roman numerals to the right. Each rectangle has on the top line five squares representing the five types of carapace described in Table I and on the bottom line four squares representing the four types of head pattern in Table II. The first square in each line represents the extreme similar to the holotype of *K. b. palmarum*. The middle squares represent intermediates and the condition more nearly typical of northern mainland specimens in that order. The last square in each line represents specimens such as are found on the Lower Keys. Numbers in the squares are the number of individuals from the designated area falling into each category of carapace or head pattern. Locality dots are condensed along the Tamiami Trail.

bee, 2.0 along the Tamiami Trail, and 1.9 near Paradise Key, to an average of 1.8 on the Lower Keys. The situation for the other two ratios is not so clear. For the anterior lobe of plastron over width of bridge ratio, there appears to be a general increase in average value from north to south, but not in so uniform a fashion. The carapace width over anterior lobe of plastron ratio averages are irregular, with both high and low values occurring in the southern mainland areas. The small samples probably account for the irregular variation shown by the

TABLE III

Means and observed ranges of three ratios expressed as percentages: width of bridge over carapace length, anterior lobe of plastron over width of bridge, and carapace width over anterior lobe of plastron. The data are segregated by sex, and are given for each of the twelve areas shown on Map 1. No specimen under 55 mm. in carapace length was used to compute the ratios.

Areas	1	2	3	4	5	6	7	8	9	10	11	12
Number of Females	18	7	6	5	12	3	9	21	20	16	3	13
Width of bridge/ Carapace length	223 (200–244)	226 (217–243)	225 (185–244)	212 (192–236)	212 (195–226)	200 (183–216)	205 (194–215)	204 (159–241)	205 (142–238)	195 (167–232)	197 (178–212)	179 (146–209)
Anterior lobe/ Width of bridge	141 (125–164)	130 (123–143)	146 (123–185)	144 (131–169)	141 (130–154)	155 (141–165)	151 (133–175)	148 (125–185)	151 (124–213)	151 (127–179)	148 (135–174)	166 (136–204)
Carapace width/ Anterior lobe	226 (203–246)	236 (220–266)	225 (216–240)	228 (207–246)	228 (211–246)	232 (213–245)	219 (204–232)	221 (190–241)	229 (206–249)	226 (213–263)	244 (231–263)	224 (207–236)
Number of males	7	1	2	2	3	1	1	12	8	15	0	6
Width of bridge/ Carapace length	180 (158–190)	201	172 (141–203)	201 (198–204)	164 (163–167)	174	163	164 (145–183)	153 (144–174)	159 (139–174)	None	166 (151–177)
Anterior lobe/ Width of bridge	170 (159–201)	144	179 (152–206)	154 (149–159)	183 (178–193)	182	182	173 (158–207)	190 (170–207)	184 (158–197)	None	172 (163–186)
Carapace width/ Anterior lobe	230 (216–248)	238	216 (214–219)	229 (227–231)	240 (226–259)	233	217	225 (204–238)	228 (213–238)	225 (210–248)	None	223 (207–240)

averages of these ratios for the males. The males seem to follow the same trends as the females.

Our investigation indicates that two of the chief characters used by Stejneger to differentiate *K. b. palmarum* from *K. b. bauri* occur to some extent throughout the mainland populations of the species. The other characters employed by Stejneger appear to be of little use in distinguishing two mainland subspecies of *Kinosternon bauri*.

We feel that the variational patterns shown by the characters of mainland *K. bauri* are either clinal or else unimportant, and that they are insufficient to justify the naming of two subspecies on the mainland of Florida. However, the specimens from the Lower Keys are distinguishable from Upper Keys and mainland specimens. We therefore propose that the name *K. b. bauri* be restricted to turtles from the Lower Keys, and that turtles from the Upper Keys and peninsular mainland of Florida be known as *K. b. palmarum*.

Kinosternon bauri bauri Garman

Type locality: Key West, Monroe County, Florida.
Range: The Lower Florida Keys; specimens known from Key West, Stock Island, and Big Pine Key; assumed to occur on all keys of the Big Pine Key group having suitable habitats.
Diagnosis: Carapace dark with three dorsal light lines often almost obliterated by dark pigment; head skin generally very dark with head stripes prominent to almost obliterated; mandibular beak unstreaked or only slightly streaked.

At the time of the description of *K. bauri*, Garman had thirteen specimens, of which twelve were from Key West and one was purportedly from Cuba. Of these, we have seen eleven individuals, including the supposed Cuban specimen. Seven (MCZ 4380) are young (carapace lengths 22 to 46 mm.); these are all fairly dark, and with one exception have three dorsal light lines. The mandibular beaks vary from unstreaked to heavily streaked. The three adults from Key West examined agree well in carapace pigmentation with fresh specimens from the Lower Keys, and the mandibular beaks, though somewhat faded, appear to have been unstreaked. The age of these cotypes should be taken into consideration when comparisons with more recently collected material are made.

Garman stated that the "Cuban" specimen was received from Filipe Poey. Its provenance is in doubt, for the genus *Kinosternon* is otherwise unknown from Cuba. Barbour and Ramsden (1919: 76) have pointed out that Poey is also credited with a specimen of *Gerrhonotus* supposedly from Cuba. In the original description, Garman described the "Cuban" turtle as "light yellowish-olive," and stated that it had a streaked mandibular beak. On the basis of Garman's description and our examination, we feel that the "Cuban" turtle came from the Florida mainland or the Upper Keys.

That *Kinosternon b. bauri* is limited to the Lower Keys is perhaps a reflection of the historical difference between these islands and the Upper Keys. The Lower Keys represent bits of the mainland Miami Oolite Formation, while the

Upper Keys are composed of the Key Largo Limestone Formation. Both formations are probably of Pleistocene Age (Cooke, 1945: 248–265). Subspecific differentiation has occurred in the time since the Lower Keys have been separated from the mainland by the formation of the Florida Bay. Since their emergence, the Upper Keys have apparently never been connected to the mainland.

Kinosternon bauri palmarum Stejneger

Type locality: Paradise Key, Dade County, Florida.
Range: Mainland Florida from Leon and Columbia counties south to Dade and Monroe counties, and the Upper Florida Keys.
Diagnosis: Carapace variably pigmented, seldom as dark as *K. b. bauri*, with the three dorsal light lines either very obscure because of the translucent laminae, or very marked because of the dark pigmentation, but almost never obliterated due to encroachment of dark pigment; head skin variable in color and pattern; mandibular beak usually heavily streaked.

The variation of characters within the subspecies *K. b. palmarum* has already been discussed.

We wish to thank the following for loan of material: Norman E. Hartweg and William E. Duellman, Museum of Zoology, University of Michigan; Arnold B. Grobman, University of Florida; Hobart M. Smith, University of Illinois; M. Graham Netting and Neil D. Richmond, Carnegie Museum; Doris M. Cochran, United States National Museum; Arthur Loveridge, Museum of Comparative Zoology. Dr. Cochran and Mr. Loveridge have courteously allowed us to work at their respective institutions, and the former has supplied us with a photograph of the holotype of *K. b. palmarum*. We wish also to thank the many persons who, knowing our interest in the problem, have collected these turtles for us in Florida, especially L. Neil Bell and William E. Duellman.

SPECIMENS EXAMINED:—All from Florida, as follows. *Kinosternon b. bauri:* MONROE CO., Big Pine Key, 16; key 30 mi. n. Key West, 1; Stock Island, 4; Key West, 11. *Kinosternon b. palmarum:* ALACHUA CO., Gainesville, 3; 6 mi. s.w. Gainesville, 1; Micanopy, 1; near Lochloosa, Lochloosa Lake, 2; Payne's Prairie, Biven's Arm, 1; no other locality, 15. PUTNAM CO., Welaka, 1. LEVY CO., 2.5 mi. s.w. Bronson, 1. MARION CO., Burbank, 1. ST. JOHNS CO., 2. VOLUSIA CO., Lake Helen, 2; 2 mi. w. DeLeon Springs, 1; 6 mi. s.e. Deland, 1; 11 mi. s.w. Daytona Beach, 1. ORANGE CO., Orlando, 2. BREVARD CO., 12 mi. e. Indian Run and 0.5 mi. e. St. Johns River, 1. CITRUS CO., 4 mi. w. Dunnellon 1; 2.2 mi. e. Inverness, 1. LAKE CO., Lake Yale, 2; Leesburg, 1; Emeralda, 3. HERNANDO CO., Brooksville, 1. HILLSBOROUGH CO., Plant City, 1. POLK CO., Winter Haven, 1; no other locality, 2. PINELLAS CO., 1 mi. e. Dunedin, 1; near Gulfport, 1; Safety Harbor, 1. SARASOTA CO., 6.1 mi. n.w. Englewood, 1; 7 mi. n., 4.5 mi. e. Englewood, 1. CHARLOTTE CO., Englewood, 2. OKEECHOBEE CO., Okeechobee, 1. MARTIN CO., 2.3 mi. n. Pt. Mayaca, 1. PALM BEACH CO., 11 mi. w. Lantana, 1; Lake Okeechobee, 2. GLADES CO., 21.3 mi. s.w. Okeechobee, 2; 22.1 mi. s.w. Okeechobee, 1; 22.3 mi. s.w. Okeechobee, 1; 9.2 mi. n.e. Indian Prairie Canal, 1; 11.7 mi. n.e. Lakeport, 1; 7.2 mi. n.e. Lakeport, 2; 3.3 mi. n.e. Lakeport, 1; 2.4 mi. n.e. Lakeport, 1; 18 mi. n. Moorehaven, 1. BROWARD CO., 21.5 mi. n.w. Ft. Lauderdale, 1; South New River Canal at Miami-Okeechobee Rd., 1; 4.6 mi. s. junction Ft. Lauderdale-Okeechobee Rd., 1; 1 mi. n. Dade Co. line, 1. COLLIER CO., 0.9 mi. s.e., thence 0.4 mi. n.e. Naples, 1; between 2.7 and 13.5 mi. s.e. Royal Palm Hammock, 7; 2 mi. e. Everglades, 1; between 4.1 and 10.8 mi. w. Carnestown, 8; 8.2 mi. w.

Ochopee, 1; 3.7 mi. w. Ochopee, 1; 1.5 mi. w. Ochopee, 1; Ochopee, 2; 0.5 mi. e. Ochopee, 1; 8.2 mi. w. Monroe Station, 1; 12.6 mi. e. Monroe Station, 1; 19.6 mi. e. Monroe Station, 1; center of Everglades, 1; Thickahatchee Swamp, 1. DADE CO., between 12 mi. w. and 19 mi. w. Miami, 6; between 20 mi. w. and 27.9 mi. w. Miami, 19; between 30 mi. w. and 39.3 mi. w. Miami, 7; 40 mi. w., 1 mi. n.w. Miami, 1; 19 mi. w., 6 mi. s. Miami, 1; 19 mi. w., 3 mi. s. Miami, 1; 12 mi. w., 1 mi. n. Miami, 1; 12 mi. w., 2 mi. n. Miami, 1; 7 mi. w., 2.5 mi. n. Miami, 2; 2 mi. n.w. Miami, 2; 1 mi. n.w. Medley, 1; 4.1 mi. s. Florida City, 22; Paradise Key, 7; 0.75 mi. e. Paradise Key, 3; 1.5 mi. w. Paradise Key, 1; 17.4 mi. s.w. Paradise Key, 1; 6 mi. s. Paradise Key, toward Cape Sable, 2; no other locality, 2. MONROE CO., Upper Matecumbe Key, 1; Lower Matecumbe Key, 1; Key Vaca, 1. "Cuba", 1.

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THE GENUS *XYRIS* L. IN NORTH CAROLINA

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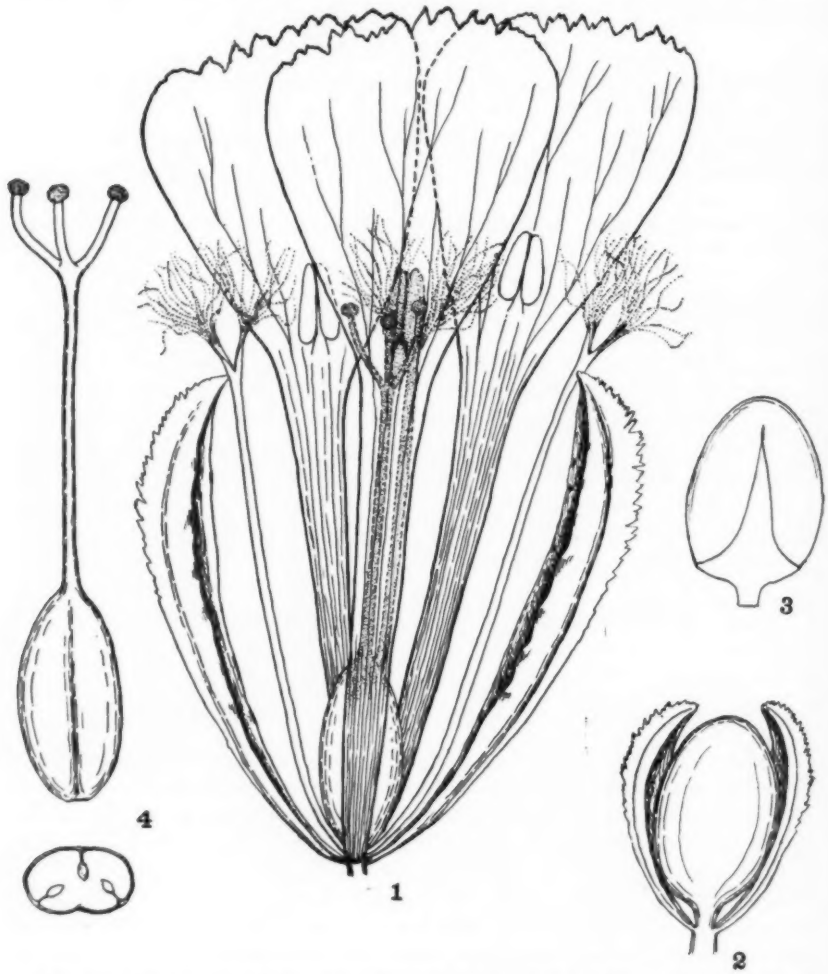
The greatest contribution to the genus *Xyris* of North America was made by Chapman, who described nine new species in the first edition of his *Flora of the Southern States* (1860). Except for the reduction of one to synonymy and another to varietal rank, Chapman's species have been accepted up to the present time. The first comprehensive treatment dealing exclusively with the genus for North America was that of Ries (1892). He reported fourteen species of which one was recognized as new (*X. montana*). The last was that of Malme (1937b). This was published posthumously with a few nomenclatural changes made by the editors. Malme had previously published on *Xyris* of North America (1913, 1937a) as well as of Cuba (1925) and South America (1933). Apparently he never visited North America and, therefore, studied only herbarium specimens. Since he did

not annotate specimens which he considered correctly determined, there is no way of knowing how much herbarium material he actually examined. Indications are that these examinations were far from exhaustive, and for some species he admitted he had not seen the types. He reported twenty-eight species and five varieties for this continent. Since that time the genus has been treated in revised regional floras, and two new species have been described. Although there is general agreement in these floras as to concepts and nomenclature, certain dif-



FIGS. 1–4. Generalized diagrammatic drawings of parts of the flower of *Xyris*. FIG. 1. Flower without antical sepal. FIG. 2. Flower bud from antical view. FIG. 3. Antical sepal viewed from inside. FIG. 4. Pistil and section of ovary below.

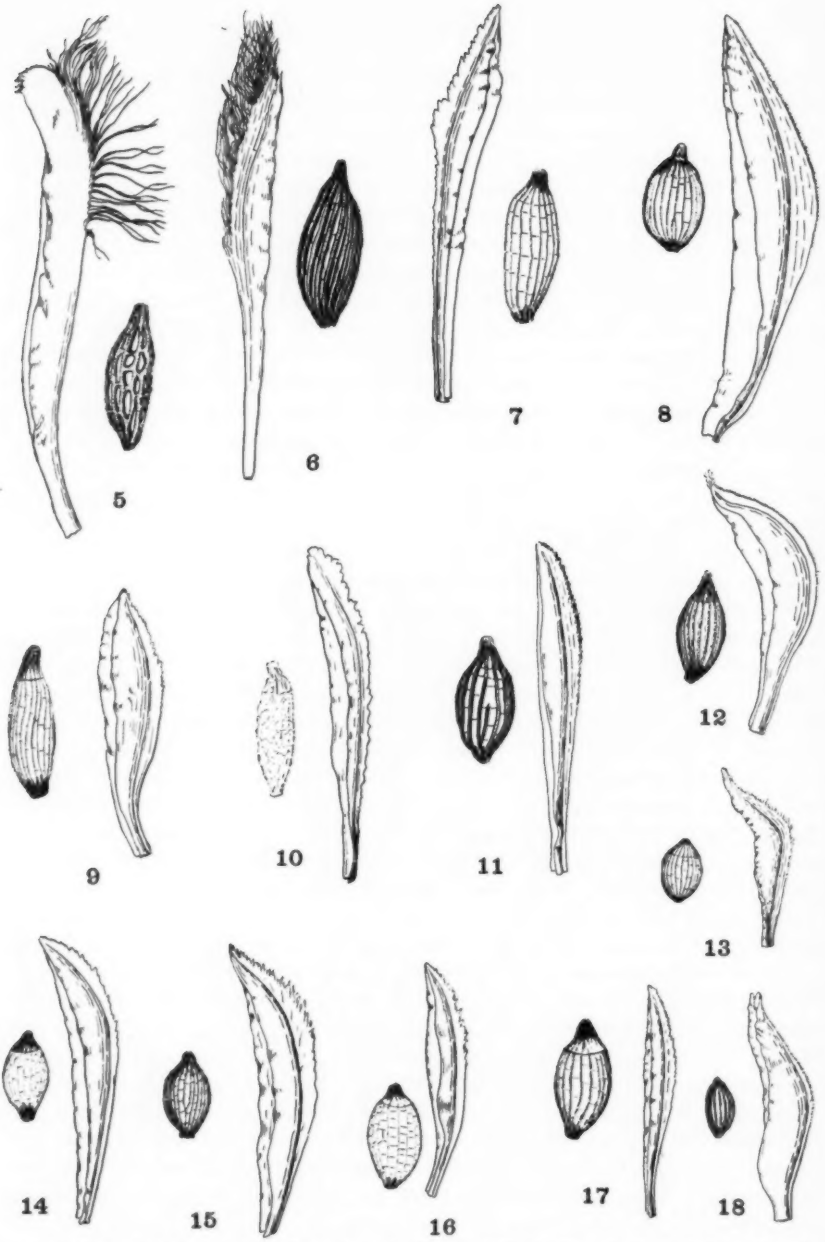
ferences are evident. Furthermore, the distribution data are sometimes incomplete as well as erroneous owing to misidentifications.

The only comprehensive treatment of the genus specifically for North Carolina was that of Curtis (1867), who listed eight species for the state. In Small's last edition of his *Manual of the Southeastern Flora* (1933), three species are stated to reach their northern range limits in North Carolina and for six others this state is included in their respective ranges. In Malme's distribution data, five are definitely reported from the state while the ranges of seven more include this area.

In the present study fourteen species and one variety have been found to occur in North Carolina. Except for one species (*X. torta* Smith), they occur mostly in the Coastal Plain where they grow in moist to wet, boggy situations and are especially abundant in man-made depressions, such as shallow excavations and ditches. Of these, two species (*X. flabelliformis* Chapm. and *X. iridifolia* Chapm.) and one variety (*X. Baldwiniana* var. *tenuifolia*) are here reported for the first time from the state. And, incidentally, in examining specimens from outside North Carolina, the known range limits of a few others have been extended elsewhere. Since this study is not a monographic one, few revisions of concepts and nomenclature have been attempted in this report, although the notes will indicate where such will no doubt eventually become necessary.

The genus *Xyris* consists of a distinctive, natural group of taxa which show a considerable degree of specialization. They have in general a rather sparse fibrous root system, basal leaves with individual sheaths and a naked flowering stem (scape or "peduncle"), except for a sheath at base, which usually overtops the leaves. The flowers are borne in a terminal, relatively short, scaly spike (rarely 2) ranging in shape from short-obovate, ovate or ellipsoidal to ellipsoid-cylindric. One flower is borne in the axil of each scale, except in a few scales at the base of the spike, which are sterile. The flowers have 3 sepals, 2 of which (the lateral) are flattened and boat-shaped with a prominent keel on the back, more or less curved, scarious and persistent (Fig. 1). The third, or outer sepal, is very different from the lateral (Figs. 2, 3), being hood-shaped and enclosing the inner parts of the flower in the bud. As the flower expands, this sepal is raised from its attached position and promptly shed. The 3 yellow petals (rarely white) have long claws which broaden above into spatulate blades, more or less erose on the outer margin. Three functional stamens are attached inside the petals nearly the whole length of the filaments. Alternating with the petals are 3 staminoids with slender filaments, flattened and bifid above and ending in numerous slender hairs, except in *X. Baldwiniana* in which these hairs are reduced to short papillae. The hypogynous pistil is 3-carpellary with a 1-celled ovary with 3 parietal placentae and numerous ovules (Fig. 4). A long style divides above into 3 branches each terminated by a capitate stigma. The fruit is a somewhat flattened capsule. The seeds are relatively small, not exceeding 1 mm. in our species, ellipsoidal to fusiform and distinctly ribbed lengthwise on the outside, the ribs joined by faint to prominent cross-ribs. In some species the seeds are covered by a powdery substance.

The flowers in the different species of *Xyris* are remarkably uniform and, therefore, offer few characters as a basis for classification. The only parts which have



FIGS. 5–18. Lateral sepals (×9) and seeds (×31) of *Xyris*. FIG. 5. *X. fimbriata*. FIG. 6. *X. flexuosa*. FIG. 7. *X. Smalliana*. FIG. 8. *X. ambigua*. FIG. 9. *X. Baldwiniana*. FIG. 10. *X. iridifolia*. FIG. 11. *X. platylepis*. FIG. 12. *X. torta*. FIG. 13. *X. flabelliformis*. FIG. 14 *X. Jupicai*. FIG. 15. *X. difformis*. FIG. 16. *X. caroliniana*. FIG. 17. *X. Curtissii*. FIG. 18. *X. brevifolia*.

been considered to be of fundamental importance are the lateral sepals. Although these vary more or less in size and shape in the different species, it is the keels which have been most emphasized. While the latter are fairly constant for each species, they exhibit some variation in populations of one taxon, as well as the variation due to different stages of development. Hence, only mature sepals should be used. However, some difficulty is encountered in the use of lateral sepals in a few species in which they are very similar. Also, it must be admitted, it takes considerable experience to distinguish "cross-toothed or incised" from "erose" or "lacerate" or "lacerate-fimbriate", descriptive terms commonly used in manuals. The seeds are quite distinctive for most species (Figs. 5–18), but in some, which differ in other respects, the seeds are more or less indistinguishable (Figs. 14–17). The number of ribs of the scape is an obvious and fairly constant character although this may vary at different positions, and some species which are said to be "bicostate" (i.e. 2-ribbed) may have 3 ribs or only 1. The degree of scabrousness of the ribs of the scape has apparently not always been correctly interpreted. In some species, such as *X. fimbriata* and *X. platylepis*, scabrousness of the ribs is obvious to both touch and unaided vision; but in others, such as *X. Jupicai* and *X. difformis*, in which they have usually been described as "smooth", they are minutely tuberculate but may not be noticeably scabrous to touch. The seasonal habit, whether annual or perennial, is sometimes difficult to determine and should, therefore, not be used as a primary diagnostic character. There is some evidence that in certain species, such as *X. Jupicai* of our area, some plants of one population may be annual while others are perennial. However, where perennating structures, such as bulbous bases or root-crowns, are present it may be safely assumed that the plants are perennial.

KEY TO SPECIES

1. Keel of lateral sepals divided above into long fimbriae which project above the upper margin and sides of the subtending floral bract.
   2. Fimbriae light-brown, not much entangled or twisting; scapes several-ribbed, at least in the middle, the ribs markedly scabrous; leaves 6–10 mm. broad, not habitually twisting; plants neither deep-rooted nor castaneous at base.....1. *X. fimbriata*
   2. Fimbriae reddish-brown, usually much entangled and/or twisting; scapes 1–2-ribbed, the ridges slightly scabrous; leaves not more than 4 mm. wide, flexuous and twisting; plants deep-rooted and castaneous at base.....2. *X. flexuosa*
1. Keel of lateral sepals not divided into long fimbriae, but ciliate-scabrous or more or less erose, lacerate, lacerate-fimbriate or irregularly dentate.
   3. Lateral sepals projecting above the upper margin of the subtending bracts; seeds relatively large (about 0.8 mm. long).....3. *X. Smalliana*
   3. Lateral sepals not projecting above the upper margin of the subtending bracts.
      4. Keel of lateral sepals ciliate-scabrid from near the base, or the middle, to the apex.
         5. Upper flowering bracts usually short-fimbriate on the upper margin; lateral sepals with a tuft of hairs at the apex; leaves narrow, 1–2.5 mm. broad, and usually conspicuously twisting.....4. *X. torta*
         5. Upper flowering bracts not fimbriate on the upper margin which may be entire or more or less lacerate; lateral sepals not bearded at apex.
            6. Plants relatively large, the scapes 50–100 cm. tall, perennial; leaves 3–5 mm.

broad and 15–40 cm. long, closely imbricated and prominently distichously arranged (fan-shaped) at base.....5. *X. ambigua*

6. Plants relatively small, the scapes rarely more than 30 cm. tall, annual; leaves linear, not more than 2 mm. wide and 2–6 cm. long, not fan-shaped at base.
   7. Flowering bracts (especially the upper) more or less lacerate on the upper margin; leaves 3–6 cm. long, not habitually curved, smooth; lateral sepals only slightly falcate.....6. *X. brevifolia*
   7. Flowering bracts entire or nearly so on the upper margin; leaves 1–3 cm. long, often curved, the outer papillose in transverse lines on the back; lateral sepals strongly falcate.....7. *X. flabelliformis*

4. Keel of lateral sepals not ciliate-scabrid, but erose or more or less lacerate or irregularly dentate.
   8. Leaves filiform, terete or slightly flattened, not more than 2 mm. wide; the flowering bracts conspicuously lacerate; staminoids not fimbriate at apex.
      9. Leaves terete, 0.3–0.5 mm. thick.....8. *X. Baldwiniana* var. *Baldwiniana*
      9. Leaves flattened, 0.6–2 mm. broad.....8a. *X. Baldwiniana* var. *tenuifolia*
   8. Leaves not filiform, always flat and more than 2 mm. wide (except in juvenile or depauperate specimens); staminoids long-fimbriate at apex.
      10. Lateral sepals dark-brown to purplish, with a narrow keel lacerate from near the base to apex; seeds narrowly fusiform and farinose when mature 9. *X. iridifolia*
      10. Lateral sepals not colored as in the above with mostly a broader keel, especially above, usually not much lacerate or dentate below the middle; seeds ellipsoidal, not farinose.
         11. Scapes with more than 3 ribs (multicostate) below or throughout; ribs usually scabrous.
            12. Plants distinctly bulbous, perennial, relatively tall; scapes 50–100 cm. or more tall, several-ribbed, especially below, the ribs scabrous; leaves 5–10 mm. broad, *the outermost reduced to pointed sheaths*; flowering bracts very broad and usually conspicuously veiny; lateral sepals narrowly lanceolate, the keel narrow below, broadening and shallowly lacerate above.....10. *X. platylepis*
            12. Plants not bulbous, annual, small; scapes not more than 40 cm. tall in our area, *twisting and prominently few-ribbed* (often 4–5), the ribs usually markedly scabrous (rarely smooth); leaves linear, 2–4 mm. broad and 5–10 cm. long, the outer usually tuberculate on the back; flowering bracts not broad; lateral sepals straightish with a narrow keel erose above or at the apex only.....11. *X. Curtissii*
         11. Scapes mostly 2-ribbed (bicostate), the ribs minutely tuberculate or smooth.
            13. Leaves broadly to narrowly linear-lanceolate, 3–14 mm. broad and 20–35 cm. long, tapering at both ends; *scape strongly winged above*, the ribs usually minutely tuberculate; keel of lateral sepals broad and lacerate or lacerate-fimbriate above the middle; seeds 20–25-ribbed.....12. *X. difformis*
            13. Leaves linear to narrowly linear-lanceolate, 2.5–3.5 mm. broad; scape not strongly winged above, the ribs minutely tuberculate or smooth; keel of lateral sepals narrow to broad and irregularly dentate or lacerate to lacerate-fimbriate; seeds about 15–20 ribbed.
               14. *Ribs of scape tuberculate*; spikes ovoid to ellipsoid, usually many-flowered (except in juvenile specimens); flowering bracts closely imbricated, mostly entire to slightly lacerate on upper margin; keel of lateral sepals rather narrow, irregularly dentate to erose

above the middle (rarely almost entire); seeds with 18–20 ribs, about 0.4 mm. long.....13. *X. Jupicai*

14. *Ribs of scape smooth*; spikes short-obovate to ellipsoid, usually few-flowered, the flowering bracts loosely imbricated, the upper more or less lacerate on upper margin; keel of lateral sepals broad above and lacerate to lacerate-fimbriate above the middle; seeds with about 15 ribs, 0.5 mm. long.....14. *X. caroliniana*

LIST OF SPECIES

For conservation of space in publication, synonymy has been reduced in this report to only those names in Small's *Manual of Southeastern Flora* (1933) and Fernald's *Gray's Manual of Botany* (1950), which have been misapplied, are homonyms, or represent groups which have been merged with other taxa. For the same reason, citation of specimens is limited to those taxa which have been collected only a few times or reported for the first time from North Carolina. For those of more frequent occurrence, the distribution is indicated on spot-maps.

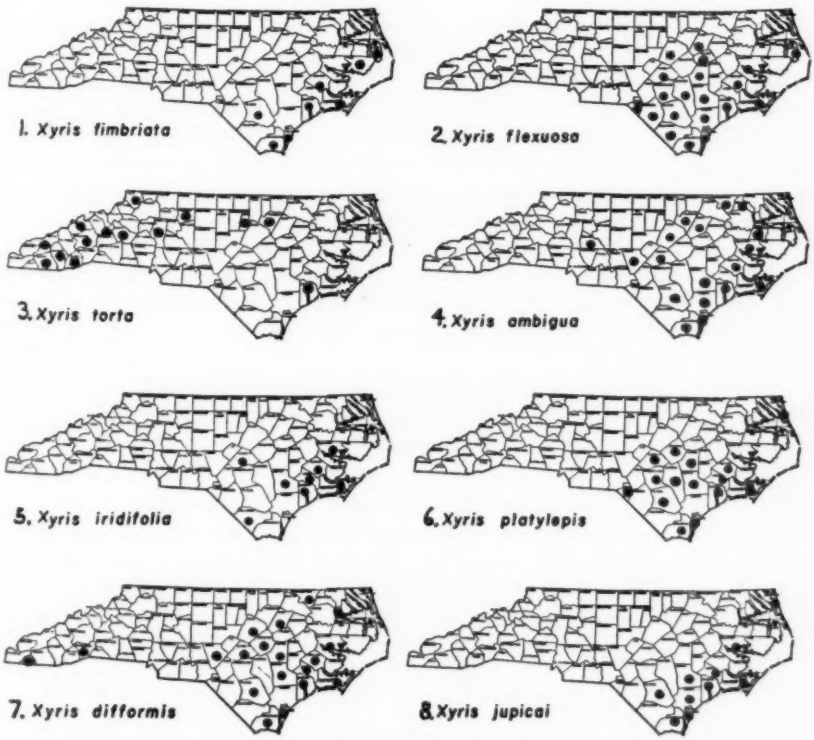


FIG. 19. Distribution maps of 8 species of *Xyris* in North Carolina

1. *X. fimbriata* Ell. Fig. 5. Map. 1.

One of the most easily recognized species by the scabrous many-ribbed scape and the exposed fimbriae of the keels of the lateral sepals. A robust perennial growing in wet, boggy situations. Collected only in the coastal counties in N. C. General distribution: Fla. to Miss., n., in the Coastal Plain, to N. J.

2. *X. flexuosa* Muhl. *ex.* Ell. Fig. 6. Map 2.

Another easily distinguished species by its narrow (1–3 mm.), erect, twisted and flexuous leaves and the deep-rooted, bulbous, castaneous base, as well as the long, narrow, pointed spikes with the exposed, reddish, slender, entangled fimbriae of the keels of the lateral sepals. This species grows in drier situations than most of the other species found in the state. Low pine barrens, often in sandy soil. Frequent throughout the Coastal Plain, except in the northern counties. General distribution: Fla. to Tex., Ark., n., in the Coastal Plain, to N. J.; also in Cuba.

Note: The white-flowered form (*X. flexuosa* var. *pallescens* (Mohr) Barnh.), which seems to be fairly frequent further south, has not, so far as known, been collected in N. C.

3. *X. Smalliana* Nash. Fig. 7.

Not easily distinguished superficially from some of the other species, except by its broadly linear, relatively long leaves, and the exposed tips of the lateral sepals. However, the exposure of the latter varies considerably so that in some specimens they are barely visible above the upper edge of the floral bracts. Its seeds are relatively large, about 0.8 mm. in length, with few widely spaced ribs. Rather infrequent in the outer southeastern Coastal Plain. Bladen Co.: *Buell Oct. 26, 1941* (NCS); Brunswick Co.: *Blomquist and Godfrey 15212* (DUKE); Carteret Co.: *Blomquist 16163* (DUKE); Craven Co.: *Blomquist 16197* (DUKE); New Hanover Co.: *Godfrey and Wells 6204* (NCS, GH); Richmond Co.: *Wiegand and Manning 699* (CU). General distribution: Fla. to La., n., in the Coastal plain, to N. C.

Note: This species is difficult to distinguish from the more northern *X. Congdoni* Small, which, it seems, should be considered as only a variety of the above (*X. Smalliana* var. *Congdoni* (Small) Malme).

4. *X. torta* Smith (*X. flexuosa, non* Muhl.) Fig. 8. Map 3.

This is the only species which is at all frequent and widely distributed in the western, mountainous part of the state, but does extend to the lower Piedmont. A single collection from Onslow Co. of the outer Coastal Plain may be an error in labelling. In moist, boggy situations. General distributions: Widely distributed inland from Ga. to Ark. and Tex., n. to N. H., w. to O. and Minn.

5. *X. ambigua* Beyr. (*X. stricta* Chapm.) Fig. 9. Map 4.

Often recognized superficially by its broad, fan-shaped base, many-ribbed scape, scabrous below, and large, ellipsoid-ovate spikes with tightly imbricated,

broad bracts. Easily identified by its large lateral sepals with ciliate-scabrous keels. Mostly a robust perennial, but variable in size depending upon habitat. Moist to dry, acid, often sandy soils. One of the most frequent species throughout the Coastal Plain. General distribution: Fla. to Tex., n., in the Coastal Plain, to s.e. Va.; also in Cuba and C. Amer.

6. *X. brevifolia* Michx. Fig. 18.

One of 2 of our smallest species. The other is *X. flabelliformis*. These are often confused and, therefore, not infrequently mounted together when collected where they happened to be growing mixed, which apparently they often do. Both are annuals, at least in our area. Superficially, they are best distingusihed by the lacerate upper floral bracts of *X. brevifolia* and the entire bracts of *X. flabelliformis*. The former has been collected only in the southeastern part of the state in moist, acid sterile soil in natural depressions or shallow ditches. Probably of wider distribution but easily overlooked because of its small size. Brunswick Co.: *Godfrey 49164* (DUKE, NCS), *Blomquist et al. 16058* (DUKE); Pender Co.: *Wells April 17, '25* (NCS). General distribution: Fla. to N. C.

7. *X. flabelliformis* Chapm. Fig. 13.

Distribution and habitats in the state about the same as No. 6, but has been collected further north. Sometimes exceedingly small and easily overlooked. Brunswick Co.: *Blomquist et al. 16058A* (DUKE); Carteret Co.; *Blomquist and Rock 16418* (DUKE). General distribution: Fla. to Miss. and N. C.

8. *X. Baldwiniana* Schult. in R. & S. var. *Baldwiniana*. Fig. 9.

A deep-rooted, small to medium-sized perennial with erect filiform, terete leaves and broadly ovate spikes in which the upper bracts are conspicuously lacerate. The only species in the state without long-fimbriate staminoids. The seeds are relatively large, about 0.8 mm. long. Wet pine barrens and boggy situations in the southeastern Coastal Plain. Bladen Co.: *Heller 14016* (F); Carteret Co.: *Blomquist 15592A* (DUKE); Columbus Co.: *Blomquist et al. 16028* (DUKE); Cumberland Co.: *Bilt. Herb. 4799f* (GH); New Hanover Co.: *Druchell 10061* (NY); Pender Co.: *Channell and Blomquist 1723* (DUKE). General distribution: Fla. to Tex. and N. C.

8a. *X. Baldwiniana* Schult. in R. & S. var. *tennifolia* (Chapm.) Malme.

This variety includes forms with more or less flattened leaves. Just how these forms are related to the type variety is unknown. Malme expresses the opinion that they are of hybrid origin from natural crosses between the typical form and *X. Elliottii* Chapm. In N. C. var. *tenuifolia* has a scattered distribution and occurs further inland than the type variety. Low pinelands and boggy situations: Coastal Plain and Piedmont. Carteret Co.: *Channel and Blomquist 1892* (DUKE); Harnett Co.: *Channell 2862* (DUKE); Iredell Co.: *Radford and Radford 2639* (NCU); Pender Co.: *Channell et al. 2905* (DUKE). General distribution: Fla. to Tex. and N. C.

9. *X. iridifolia* Chapm. Fig. 10. Map 5.

A robust perennial, recognized by its reddish base, bicostate, smooth scape and large ellipsoid spikes which at maturity show a purplish coloration under the margin of the bracts. It is the only species in the state with farinose seeds. In various wet, boggy situations but frequent in roadside ditches of the southern Coastal Plain. General distribution: Fla. to Tex., n., to Tenn. and N. C.

10. *X. platylepis* Chapm. Fig. 11. Map 6.

A robust, bulbous perennial in which the outermost, opposite leaves are reduced to pointed sheaths. The specific epithet undoubtedly refers to the extremely broad bracts. Moist to wet pinelands, swamps and ditches, throughout the coastal Plain. General distribution: Fla. to La., n., in the Coastal Plain, to s.e. Va.

11. *X. Curtissii* Malme (*X. neglecta* Small, *non* A. Nilss., *X. Bayardii* Fern.) Fig. 17.

A rather small annual in our area. Many specimens from further south are generally taller and may be perennial. Easily recognized by its short linear leaves the outer of which are tuberculate on the back, and the few-ridged (usually scabrous), twisted scape. Apparently rather infrequent in N. C. Coastal Plain to lower Piedmont. Carteret Co.: *Blomquist 15592* (DUKE); Moore Co.: *Katzenstein Sept. 15, 1897* (GH); Wake Co.: *Godfrey 4986* (DUKE, G, NCS). General distribution: Fla. to Miss., n. in the Coastal Plain to Va.

Note: *X. Bayardii* Fern. of which we have examined the type material seems to be only a small form of *X. Curtissii*.

12. *X. Difformis* Chapm. Fig. 8. Map 7.

This appears to be a distinct species although Malme expressed the opinion that it may be only a variety of *X. caroliniana* Walt., as generally understood. It has a perennial, tufted habit with usually some (at least the outer) broad linear-lanceolate, ascending leaves which taper to both ends, often reddish at base. However, narrow-leaved forms occur resembling *X. caroliniana* on the one hand and *X. Jupicai* on the other. From the former such may be distinguished by the strongly winged scape below the spike, which is usually slightly tuberculate on the ridges, and the smaller seeds with more ridges. The distinction between these two in the comparative translucency of the dried leaves, used by Fernald in the 8th edition of *Gray's Manual*, is too indistinct to be relied upon, as well as impractical in examining mounted specimens. From *X. Jupicai*, it may be distinguished by its tufted habit, longer, more ascending leaves and winged scape, and from both by its more dentate than lacerate keel of the lateral sepals. Moist, peaty situations, throughout the coastal Plain, and upland bogs in the southwestern part of the state where only narrow-leaved forms have been collected. General distribution: Fla. to Okla. and e. Tex., n., to N. J.

13. *X. Jupicai* Rich. (*X. elata* Chapm., *X. communis* Kunth). Fig. 14. Map 8.

After examining the type of *X. elata* Chapm. and a cotype of *X. Jupicai*, as well as many specimens from various provinces assigned to these 2 species respectively, we have arrived at the conclusion that they are indistinguishable and should be merged into one taxon. This appears to have been the opinion of Malme also, who says of *X. elata:* "Note: Scarcely distinct from *X. Jupicai*, which is not mentioned by Chapman." This is a wide-ranging species, occurring from northern South America, through Central America and the West Indies, and from Fla. to Tex. and Okla., n., to Tenn. and Va. In N. C. it is frequently a pioneer on road shoulders, in shallow ditches and other excavated areas. As such, it appears to be mostly an annual and is extremely variable in size even in the same population. However, further south, it seems to be perennial. Forms of this species have undoubtedly been confused with both *X. caroliniana* and *X. difformis*. It differs from both in the less tufted habit, short, erect, linear leaves and the tuberculate ridges of the scape. Its seeds are more like those of *X. difformis* than *X. caroliniana*. The keel of the lateral sepals is quite distinct from those of the latter two. Very common in the outer Coastal Plain.

14. *X. caroliniana* Walt. Fig. 16.

Since there is no type specimen of this species in Walter's herbarium and his description (1788, p. 69) is so brief that it could include several other species, it is very uncertain to what plants this name should apply. According to Elliott (1821, p. 59), it was *X. flexuosa* Muhl., but there is no evidence that he had seen Walter's plant. The only part of Walter's description which might be said to be specific is: "Fol. gladiatis, scapo longa, . . .". This would at least indicate that it was not *X. flexuosa*, which has linear, twisted leaves. Neither does it apply to what has been considered to be *X. caroliniana* in which the leaves are also linear. Furthermore, the latter, as generally understood, seems to be very rare, if present at all, in S. C. where the type was supposed to have been collected. It, therefore, seems strange that Walter should have collected this rather than some common species. It is more likely that it represented *X. difformis*, the typical forms of which have linear-lanceolate leaves, and which is fairly frequent in that state. Two specimens which agree fairly well with what is considered to be *X. caroliniana* in the northeastern states have been collected in the outer Coastal Plain. Duplin Co.: *Channell and Blomquist 1811B* (DUKE); Onslow Co.: *Channell and Blomquist 1810B* (DUKE). Many specimens from this state in various herbaria, which have been identified as this species, are mostly *X. difformis* or some other species. Because of the uncertain status of this species, as well as its having frequently been confused with other species, its general distribution is at present uncertain. The disposition of it will have to await further study.

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ACINETOSPORA BORN. NEW TO NORTH AMERICA

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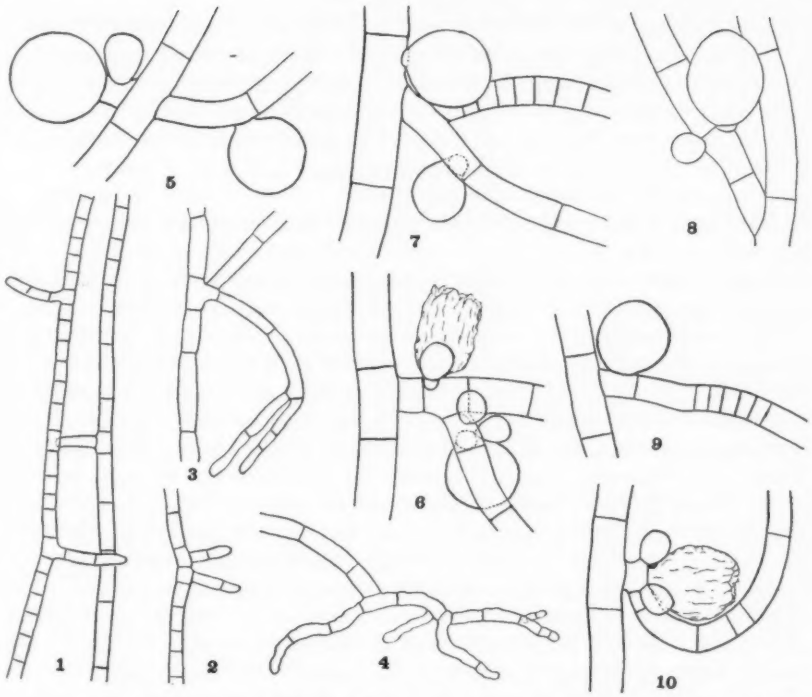
In a study of the marine algae growing on a rock jetty at Cape Lookout, North Carolina, Williams (1948) reported the occurrence of a surprisingly large number (60) of taxa new to this area. Except for a new species of *Oscillatoria* (*O. Williamsii* Drouet), most of these records represented northern or southern extensions of ranges. However, five European species were reported for the first time from the Western Atlantic Ocean. To these can now be added another one, which was erroneously reported—although with some uncertainty—as *Ectocarpus breviarticulatus* J. Ag., a species of tropical affinities. Recently I have had the opportunity of examining a preserved collection of the alga on which this report was based and have found it to be *Acinetospora pusilla* (Griffiths) Bornet, which, so far as I have been able to determine, has not previously been reported from North America. The specimen was collected by Williams, July 30, 1947, growing epiphytically on a species of *Polysiphonia* "in the upper barnacle zone".

Acinetospora pusilla is a light brown alga consisting of slender filaments with mostly scattered short branches of 1–5 cells perpendicular to the main filaments (Fig. 1). Occasionally two such branches are borne on adjacent cells ("gemminate") (Fig. 2). Longer branches of more or less indeterminate growth commonly occur on the lower portions of the erect filaments, and secondary branches may originate from the base of the primary ones (Fig. 3). The branches commonly originate from the median portion of the mother cells instead of from the upper end as in most of the branching filamentous algae, and these branch-bearing cells are usually shorter than the non-branching cells (Figs. 1–3). In the Cape Lookout material of this alga, the cells vary considerably in width and more so in length. A number of measurements show them to range from 15–25μ in diameter and



FIGS. 1–10. *Acinetospora pusilla* (Griff.) Born. FIGS. 1–4, × 119; FIGS. 5–10, × 275. FIG. 1. Upper portions of erect filaments. Fig. 2. Gemminate branches. FIG. 3. Long branches, the lower developing rhizoids at tip. FIG. 4. Rhizoidal branches. FIGS. 5–10. Portions of plants with monosporangia.

up to 100μ in length. Growth is intercalary, taking place mostly in the upper portions of the erect filaments (Fig. 1, left filament), but also near the base of the longer branches (Figs. 7, 9, 10). The plants are anchored to the substratum by more or less extensive rhizoidal branches (Fig. 4). These may originate secondarily from the longer branches (Fig. 3).

In Europe, *Acinetospora pusilla* has been found to be very variable. Sauvageau, who studied its variation (1895), classified the different forms into four varieties, based primarily upon its relation to substrata (whether epiphytic or endophytic) and, secondarily, largely on its mode of branching. The plants from Cape Lookout agree most closely with Sauvageau's variety *riparia*.

The reproductive organs of this alga consist of plurilocular and, rarely, monolocular sporangia, both having been found on the same individuals (Sauvageau, 1899). The former consist of relatively large locules and correspondingly large spores, which may be motile (Sauvageau, 1897) or nonmotile (Askenasy, 1867; Bornet, 1891). The spores from unilocular sporangia are similar to those from the plurilocular. Apparently sexual reproduction does not occur, since fusion of reproductive cells has not so far been observed. A third reproductive organ, which has been commonly called a "monosporangium" (Sauvageau, 1898), but also

a "pseudosporangium" by the same author (1899), or a "propagulum" (Kylin, 1917), not infrequently occurs on individuals without any other sporangia, but may, according to Sauvageau (1898), occur also on individuals with other sporangia. In the Cape Lookout plants only monosporangia are present (Fig. 5–10), as was the case with those found by Lund (1941) in Denmark. They occur singly on the main filaments or on the longer branches (Fig. 7, 9), but are more commonly grouped at the base of branches (Figs. 5, 6, 8, 10). They may be sessile or provided with a short one-celled pedicel from which secondary monosporangia may originate (Fig. 8, 10). They are uninucleate and bear a single large spore. Sauvageau (1899) cultured all three kinds of spores and found that they germinated promptly and grew into filaments like those from which they had come.

The present systematic position of *Acinetospora pusilla* is still somewhat uncertain. It was first named *Ectocarpus pusillus* by Griffiths in Wyatt's *Algae Danmoiensis*, no. 2, 1835. However, for a number of years it was confused with other species of *Ectocarpus*, but in 1891, Bornet clarified its typification. It has been found to occur in the British Isles (Harvey, 1841; Newton, 1931), in France (Bornet, *l.c.*; Sauvageau, *l.c.*, *et al.*), Spain (Sauvageau, *l.c.*), and more recently in the Canary Islands (Børgesen, 1926) and Denmark (Lund, *l.c.*). For a long time the only reproductive organs known in this alga were plurilocular sporangia. However, in 1891, Bornet found unilocular sporangia and observed that the spores from both kinds of sporangia were nonmotile. On the basis of this lack of motility of spores, he transferred it from *Ectocarpus* to a new genus *Acinetospora*, and to a new section, the Acinetosporée of the *Ectocarpaceae*. Later, it became known, however, through the work of Sauvageau (1896) and others, that the tendency of nonmotility of spores in *Ectocarpus* was not unusual and, furthermore, that spores of *Acinetospora* were occasionally found to be motile. Monosporangia in *A. pusilla* were discovered by Sauvageau (1898), and, since no such reproductive organ had previously been seen in the members of the Ectocarpaceae, the question arose as to its homology and what its presence might indicate as to the genetic relationship of *A. pusilla*. Since the monosporangium of this alga resembles a similar structure in certain members of the Tilopteridales, Sauvageau (1899) transferred it to this order. He even considered that it might be congeneric with *Heterospora Vidovichii* (Menegh.) Kuckuck, a Mediterranean alga, which had been placed in this order because it bears monosporangia. This disposition of *A. pusilla* was criticized by Kylin (1917), who maintained that, since the monospore of the latter is uninucleate, it has no relation to the Tilopteridales in which the typical monospores are 4-nucleate. His opinion was that the monospore of *Acinetospora* was simply a propagulum which reproduces the plant vegetatively and is outside the alternating life cycle. Børgesen (*l.c.*) indicated a similar opinion in retaining it not only in the Ectocarpaceae but in the original genus *Ectocarpus*. Lund (*l.c.*), however, associates it with *Haplospora* and *Tilopteris* of the Tilopteridales, as does Papenfuss (1951), who apparently also accepts Sauvageau's point of view that *Heterospora Vidovichii* belongs to the same genus as *Acinetospora*.

In conclusion, it might be said that in view of the absence of monosporangia in the genus *Ectocarpus*, it would appear that this plant should be retained in the

genus *Acinetospora*, despite its many resemblances to *Ectocarpus*. On the other hand, since there is some doubt about the homology of the monosporangium of this alga with the typical 4-nucleate monosporangium of the Tilopteridales, the life histories of which are still imperfectly known, it would seem best to retain *Acinetospora* in the Ectocarpales.

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NUCLEAR ABERRATIONS IN THE SYNCYTIAL ENDOSPERM OF *COCOS NUCIFERA*[1]

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In two recent publications (Cutter and Freeman, 1954; Cutter, Wilson and Freeman, 1955) we have called attention to the occurrence in the syncytial endosperm of *Cocos nucifera* of remarkable free cells or vesicles which float suspended in the liquid syncytium. These function in the initiation of the meristem

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which gives rise to the solid cellular endosperm of the mature coconut. We have also expressed the opinion that amitosis is the usual mode of nuclear division in the syncytial endosperm of *Cocos* although reports to the contrary have appeared (Dutt, 1953). It is our purpose here to document more fully the development of these endosperm vesicles and the mitotic irregularities which occur as they mature.

The literature on the development of the embryo sac and endosperm of *Cocos* has been reviewed by us in earlier publications (Cutter, Wilson and Dubé, 1952; Cutter, Wilson and Freeman, 1955). As yet no study of the stages in the embryo sac between fertilization and the differentiation of the liquid syncytial endosperm has appeared and we have not been able to secure adequate material of young fertilized ovules to complete such a study. However the stages prior to fertilization have been carefully investigated by Quisumbing and Juliano (1927) and the post fertilization stages after the appearance of the liquid syncytium have been extensively studied by ourselves and Dutt (1953).

METHODS

Coconuts were obtained in the vicinity of Miami and Naples, Florida, and were studied there in the fresh condition and were also shipped for further study to New Haven, Connecticut, and Greensboro, North Carolina. The behavior of the free nuclei, endosperm vesicles and endosperm meristem has been investigated with a number of the usual cytological techniques including the Feulgen reaction, erythrosin and crystal violet, and safranin and fast green. Most of the illustrations given here, however, were taken from slides prepared by two techniques which yield such excellent results that they will be described in detail. Since the free nuclei and endosperm vesicles are widely dispersed in the liquid syncytium (milk) of the coconut it was necessary to concentrate them for efficient study. This was done by decanting the milk from the nuts and allowing it to stand in a separatory funnel until the particulate material settled, or by slow centrifugation (2500 r.p.m.). In either case there was no distortion of the vesicles or nuclei compared with those observed in unconcentrated milk. After collection, the syncytial concentrate was spread evenly on a clean cover slip coated with Mayer's albumin, and the excess moisture was allowed to evaporate. In the first method, fixation was carried out in Osmic acid fumes for 2 minutes followed by 4 minutes' immersion in Carnoy's fluid. The material was then dehydrated through alcohols to water and stained in methyl green made up as follows: methyl green 0.5 gm. in 19 cc. distilled water,5 ml. 5 per cent ammonium alum and 0.5 cc. glacial acetic acid. The material was subsequently dehydrated through alcohols, cleared in xylene and mounted in neutral Canada balsam. This technique stained the nuclei a brilliant green leaving the nucleoli and cytoplasm colorless. The second method involved a modification of the Giemsa technique with fixation in acetone-free absolute methyl alcohol. The material was then transferred directly to 0.25 per cent aqueous azur II for 30–90 seconds, destained in acid water and counterstained in 0.1 per cent eosin Y until a pink color developed. The material was then dehydrated through alcohols, cleared in

xylene and mounted in neutral Canada balsam. Nuclei were stained bright blue and cytoplasm clear pink.

The differentiation of the endosperm meristem on the endothelium was studied by flooding fresh unfixed freehand sections of the endothelium with one per cent aqueous methyl green. Fresh concentrates of the liquid syncytium were also examined by bright and dark phase contrast microscopy.

OBSERVATIONS

In coconuts about 50 mm. in length the embryo sac is already a large cavity completely filled with a watery fluid (coconut milk) in which large free nuclei and cytoplasmic particles are suspended. At this stage there are no discrete cells present within the sac cavity other than those of the minute embryo; its liquid contents may therefore be considered a syncytium. The origins of the free nuclei within this syncytium are obscure. Quisumbing and Juliano (1927) report that the nucellar cells break down prior to fertilization, liberating their nuclei into the sac cavity. The primary endosperm nucleus also presumably undergoes divisions as the sac enlarges. If both these sources contribute nuclei to the syncytium then both diploid and triploid nuclei should be present at this stage. That there is variation in the ploidy of these nuclei is apparent from the fact that there are a variable number of nucleoli present in them at this stage (Fig. 1–5, 10, 11). These free nuclei are quite variable in size (10–90 μ) and shape. They are scattered at random throughout the cavity, and have membranes of considerable strength and resistance since they may be removed intact from the syncytium and transferred to water or isotonic sugar solutions without suffering visible distortion (Cutter, Wilson and Dubé, 1952b).

As the embryo sac cavity increases in size there is a gradual multiplication of the nuclei in the syncytium. We have not been able, in spite of intensive search, to detect any evidence of mitotic divisions among these free nuclei in the liquid syncytium. However, we have frequently encountered distorted or constricted nuclei which appear to be undergoing fragmentation or amitotic division (Fig. 6, 7, 8). Many of these aberrant nuclei may subsequently become pycnotic and degenerate (Fig. 9) and all of them show increased basophilia. However, it appears probable that at least some of these fragmented nuclei remain functional, as indicated by an increase rather than decrease in the total number of nuclei in the sac.

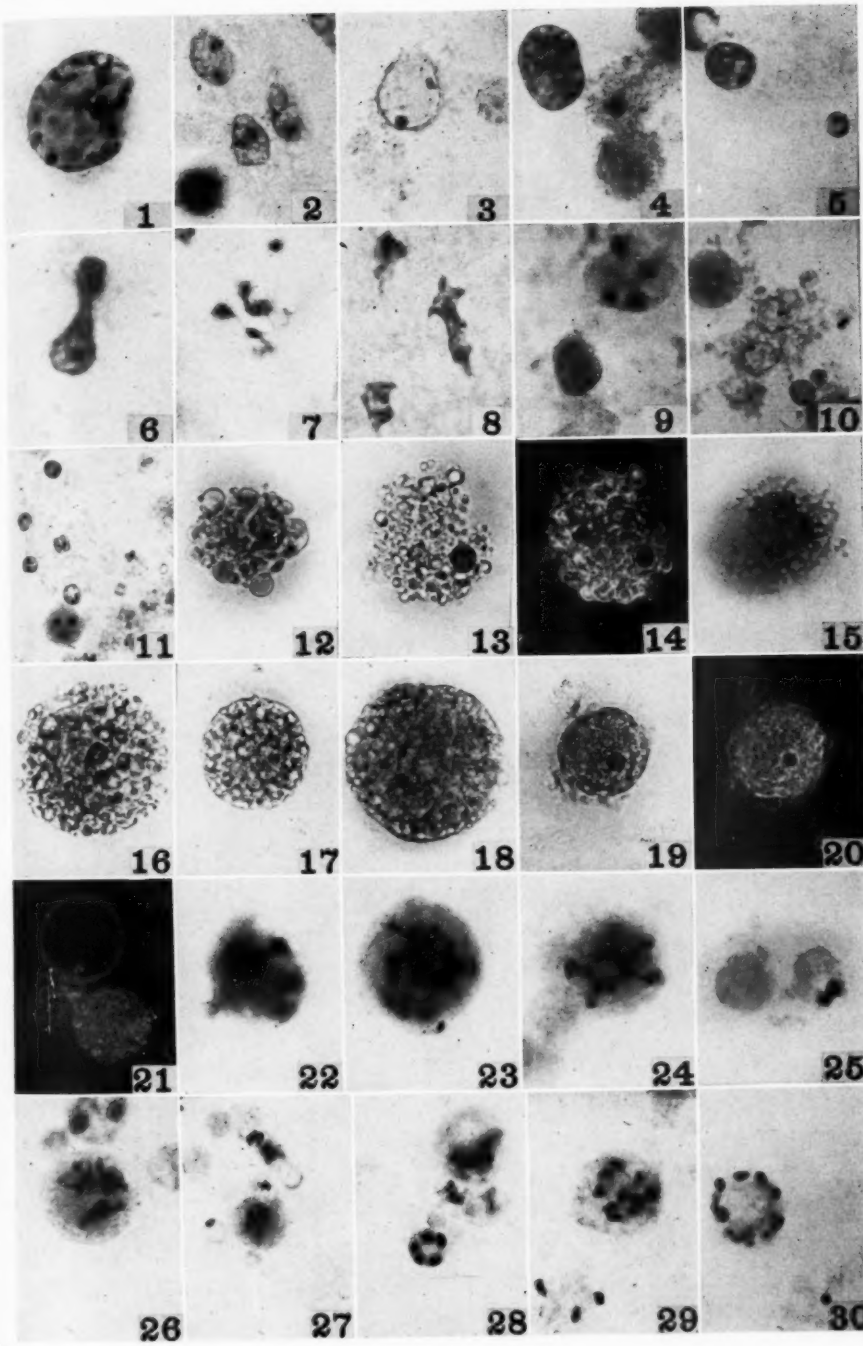
As the nut reaches an overall length of approximately 100 mm., free cells or endosperm vesicles suddenly appear suspended in the liquid syncytium. Similar endosperm vesicles have been reported in both Monocotyledons (Dahlgren, 1934) and Dicotyledons (Juliano and Alcala, 1933), but in these cases they are not as numerous or as prominent as in *Cocos*. At the time these vesicles appear there are still numerous free nuclei suspended in the milk (Fig. 10, 11). The endosperm vesicles are free spherical cells from 10–300 μ in diameter containing 1–40 nuclei. They apparently arise through coalescence of cytoplasmic materials around the free nuclei in the liquid syncytium. Their formation is apparently progressive since, at any time between their initial appearance in the

milk and their final deposition on the endothelial surface, various developmental stages may be observed. The first evidence of their formation is seen around certain free nuclei which appear to have fringes of organized cytoplasm about them. When these clumps of material are observed under the phase contrast microscope no discrete cell wall may be detected at first (Fig. 12–15), but cell wall material is soon formed in patches over the surface of the cytoplasmic mass (Fig. 16–18) and presently forms a continuous well-organized wall (Fig. 19, 20). We have evidence that either one or several free nuclei may serve as centers for the organization of these cells (Fig. 12, 15). Occasionally masses of granular cytoplasm will round up without any evident nucleus (Fig. 25, 27, 32), persist for a time and sometimes secrete a wall. These enucleate cytoplasmic masses apparently degenerate after a time leaving their rigid cell walls empty. Such empty, spherical cysts are occasionally encountered in the milk (Fig. 21).

Shortly after the free nuclei have become incorporated in vesicles they become condensed and more highly chromatic (Fig. 28–33, 47–49). The vesicles increase in size and aberrant nuclear divisions become very abundant within them. Again we have not been able to detect any mitoses or the formation of any type of achromatic spindle. It is evident, however, that these incorporated nuclei undergo some type of division or fragmentation (Fig. 22–27) and at times these divisions continue until the vesicles are packed with nuclei (Fig. 28–33). The majority of vesicles, however, contain only 1–8 nuclei at the time they are deposited on the endothelium (Fig. 57). In unusual cases one nucleus may expand without division until it fills the entire vesicle (Fig. 34).

Some of the types of aberrant nuclear behavior which suggest that amitosis occurs in these vesicles are pictured. One of the more frequent irregularities involves the formation of large vacuoles within the nuclear body (Fig. 35, 36). The same phenomenon is noted in free syncytial nuclei (Fig. 37). These vacuoles seem to balloon out and tear the nucleus into several fragments. Another frequent pattern is seen in equatorial and polar views (Fig. 40, 41) where the nuclear bodies become elongated and fragment into a number of chromosome-like portions. We have been tempted to interpret these groups of elongated fragments as metaphase plates but we have been unable to demonstrate the occurrence of spindle fibers and of characteristic prophase stages. Nuclei undergoing this sort of division seem to move directly from the interphase condition into this fragmentation pattern. The absence of normal prophases would seem to eliminate

FIGS. 1–30. All photomicrographs taken at the stated magnifications and reduced one half in printing. FIG. 1. Free nucleus from milk. ×2700. FIGS. 2–5. Free nuclei from milk. ×830. FIGS. 6–8. Free nuclei undergoing fragmentation in milk. ×860. FIG. 9. Pycnotic free nucleus. ×860. FIG. 10. Free nuclei with coalescing cytoplasmic particles and formed endosperm vesicle. ×860. FIG. 11. Portion of young gelatinous syncytium detached from endosperm meristem and endosperm vesicle. ×520. FIGS. 12–21. Stages in formation of endosperm vesicles photographed in phase contrast. Figs. 14, 20, 21 in bright contrast, the remainder in dark contrast. Fig. 21 showing empty vesicle wall. ×1320. FIGS. 22–24. Nuclear aberrations in endosperm vesicles. Azur II and eosin. ×1320. FIG. 25. Enucleate and binucleate endosperm vesicles. ×860. FIGS. 26–27. Fragmenting nuclei in endosperm vesicles. ×860. FIGS. 28–30. Endosperm vesicles showing varying numbers of nuclei. Azur II and eosin. ×860.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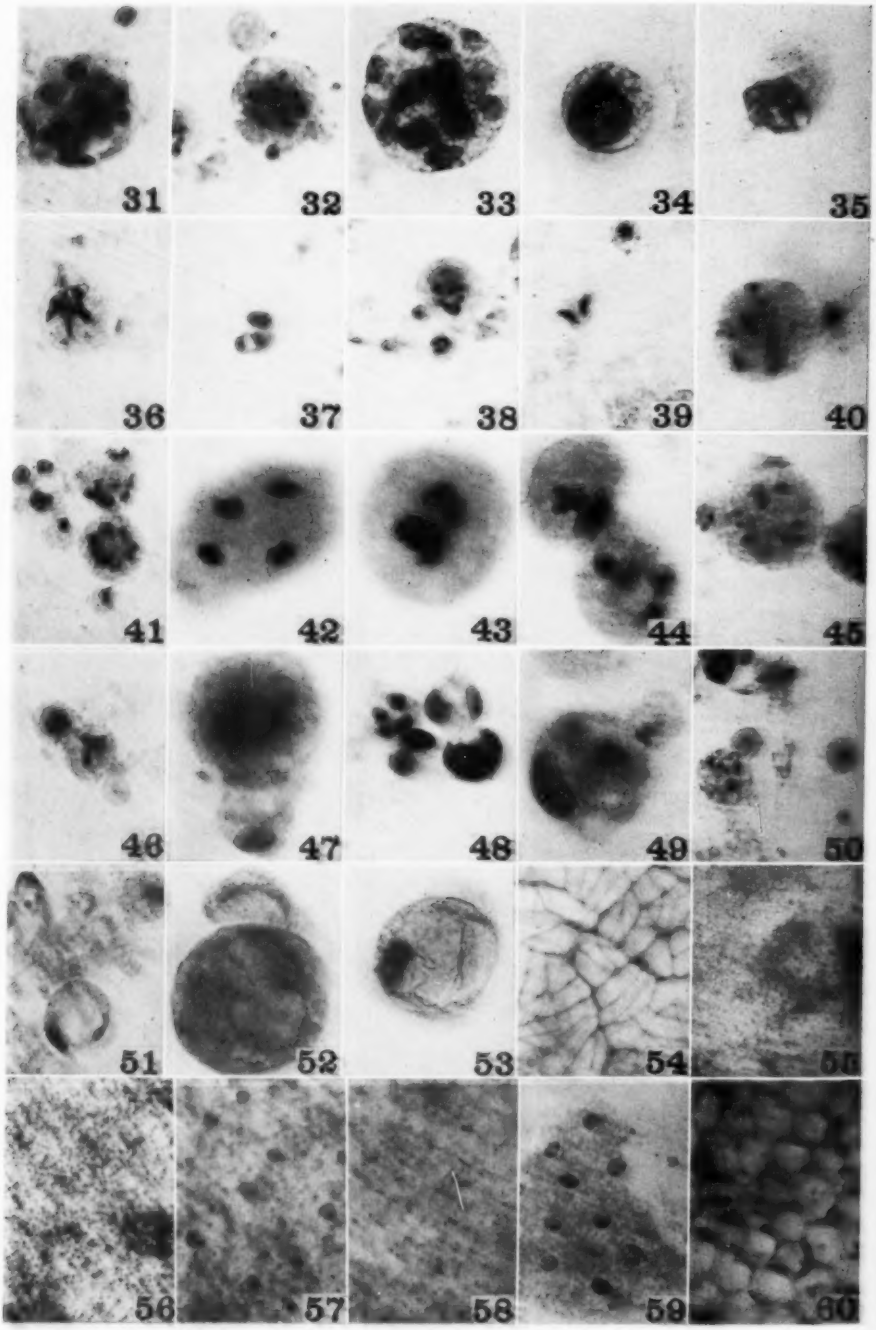
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the possibility that these divisions represent c-mitosis of the type described by Levan (1938). These nuclear fragments may further subdivide or become lobulate (Fig. 42–44) until the chromatic bodies become very minute (Fig. 45). Another characteristic division type in which the nuclear mass is separated into lens-shaped portions is represented in figures 38 and 39. Superficially such figures are suggestive of early telophase stages, but again the preceding stages have not been identifiable. The possibility that such figures represent preliminary stages in nuclear fusion has been considered but we have no further evidence that such fusions do occur. Cells are occasionally found (Fig. 51) in which large cytoplasmic vacuoles develop to the extent that the nuclei are forced against the cell wall. In extreme cases the nucleus may degenerate to leave a vacuole within the cytoplasm of the cell (Fig. 52) or the cytoplasm may degenerate leaving only the nucleus within the cell wall (Fig. 53). It is not unusual to encounter vesicles which are in the process of budding off nucleated portions which may ultimately separate as discrete cells (Fig. 46–50).

When the coconut reaches an overall length of about 150 mm. the vesicles and persistent free nuclei begin to settle out of the milk and accumulate at the lower end of the sac. As previously pointed out (Cutter and Freeman, 1954), this deposition commences at the antipodal end of the embryo sac and progresses upward around the endothelial walls until a thin gelatinous mass of cells and free nuclei lines the entire cavity, ultimately covering the embryo at the micropylar end of the sac. When the deposition begins, the endothelial wall is in direct contact with the milk (Fig. 54). The first vesicles which attach themselves are widely and rather regularly spaced (Fig. 55), but continued deposition soon results in the accumulation of a dense mass of nuclei and granular cytoplasm filled with oily materials which obscure the endothelial walls (Fig. 56, 57). At this point the cell walls of the vesicles break down, or are crushed, and the contents of the heretofore organized vesicles intermingle with the free nuclei and assorted cytoplasmic particles which have settled out of the milk, to form a dense gelatinous syncytium in which the nuclei elongate and become amoeboid in appearance (Fig. 58). Patches of this gelatinous syncytium are sometimes detached from the endothelial wall and float free in the milk (Fig. 59); in these cases the nuclei round up and again assume the characteristic appearance of the free nuclei of the liquid syncytium.

FIGS. 31–60. All photomicrographs takee at the stated magnifications and reduced one half in printing. FIGS. 31–32. Multinucleate endosperm vesicles. Azur II and eosin. ×860. FIGS. 33–34. Multinucleate and uninucleate vesicles. Feulgen reaction. ×1320. FIGS. 35–37. Nuclear vacuoles developing in free nuclei and endosperm vesicles. ×1360. FIGS. 38–41. Aberrant nuclear divisions in vesicles. Azur II and eosin. ×860. FIGS. 42–45. Lobed nuclei in vesicles. Methyl green. ×1320. FIGS. 46–50. Budding of endosperm vesicles. Figs. 46, 48, 50 ×860. Figs. 47, 49 × 1360. FIGS. 51–52. Cytoplasmic vacuoles developing in vesicles. Feulgen reaction. ×1360. FIG. 53. Uninucleate vesicle with degenerated cytoplasm. ×1360. FIG. 54. Endothelial surface prior to deposition of nuclei and vesicles from milk. ×310. FIGS. 55–56. Surface view of endothelium after deposition of nuclei and vesicles. ×150. FIG. 57. Vesicles and free nuclei deposited on endothelial surface. ×310. FIG. 58. Amoeboid nuclei in young gelatinous syncytium upon endothelial surface. ×150. FIG. 59. Free nuclei in young gelatinous syncytium. ×520. FIG. 60. Cells formed in young solid endosperm after divisions of endosperm meristem. ×310.

As this gelatinous deposition increases in thickness on the endothelium the nuclei liberated from the vesicles undergo rapid mitotic divisions, cell walls are laid down and the gelatinous syncytium is transformed into a meristem of isodiametric cells in about 7 days. During this transformation period amitotic divisions resulting in the formation of pycnotic nuclei are frequent but are supplanted by mitosis as the dominant mode of nuclear division after cell walls have subdivided the syncytium. In this conversion of gelatinous syncytium to cellular meristematic endosperm, differentiation follows the pattern established by the precipitating vesicles and nuclei in that the cellular tissue develops progressively upward around the walls of the embryo sac cavity. It is thus possible to find stages in which the young endosperm is cellular at the antipodal end of the sac, syncytial around the equatorial regions and composed of only a few scattered vesicles attached to the endothelium at the micropylar end. As a result of the greater deposition of cells and nuclei at the lower end of the sac and the greater meristematic activity in this region the cellular endosperm is always thickest here. The later stages in the development of the cellular endosperm have been described previously (Siegerman, 1951; Cutter, Wilson and Freeman, 1954).

Discussion

The development of the endosperm of *Cocos* is probably unique; the only possibly comparable report of which the authors are aware is that of Dutt (1953) for *Areca*. Although the general pattern of development follows that of nuclear endosperm as described by Maheshwari (1950), the fact that the free nuclei are suspended in a liquid rather than a gelatinous cytoplasm, and that these nuclei later become incorporated in cells, has led Dutt to state that it is impossible to classify this development pattern in any of Maheshwari's categories. Actually the proper interpretation of this type of development rests upon the nature of the liquid or milk filling the embryo sac cavity. Some authors have regarded this liquid as sap (Hill, 1937; Sampson, 1923) whereas others have considered it endosperm since it contains all the elements of a tissue although in a sol phase (Bailey, 1914). This liquid contains the usual cytoplasmic constituents including mitochondria, protein granules, oil droplets and nuclei and in all respects behaves as a liquid cytoplasm. The unusual behavior of the free nuclei in organizing particulate cellular material around themselves might be interpreted simply as the conversion of a plasma sol to a plasma gel under the influence of polymerizing enzymes elaborated by the nucleus. Since this conversion occurs first in the immediate vicinity of the nuclei which are floating free it is reasonable to expect spherical zones of gelation. Such gelation could result from pressure changes in the expanding embryo sac in a manner similar to that described by Marsland (1951) in the eggs of various marine organisms. The deposition of cell wall material upon these organized spherical masses is not essentially different from the deposition of cell wall material in the vicinity of the phragmoplasts formed in the free-nucleate gelatinous endosperm of many monocotyledons, except that the resulting cells in *Cocos*, since they are not under spatial compression, are spherical rather than many-angled.

Perhaps the most puzzling feature in the development of *Cocos* endosperm

is the apparent absence of mitotic divisions in the free nuclei and endosperm vesicles while they are suspended in the liquid cytoplasm. Dutt (1953) has reported finding nuclei in mitosis in the milk, but we have shown elsewhere (1954) that her report is probably based upon nuclei which were torn free from the endosperm meristem at a later stage of development. Marsland (1951) and Pease (1946) have shown that pressure will prohibit the formation of spindles in a sol gel system and that in all probability the spindles and their contractile fibers are properties of a gel system. While we have no data on the specific hydrostatic pressure present in the developing embryo sac of *Cocos* the system is under considerable pressure, which may be demonstrated by puncturing the sac with a hypodermic needle. Such a rupture will result in the violent expulsion of the milk. This hydrostatic pressure in the sac is reduced at the time the endosperm meristem begins to form and at this same time amitosis becomes unusual and mitosis the usual mode of nuclear division in the rapidly dividing cells of the meristem.

In explaining the frequent amitoses and fragmentations which occur in the milk nuclei and cells the possibility remains that these aberrations are the first steps in the degeneration of supernumerary diploid nuclei freed by the dissolution of the nucellar cells. However, this explanation will hardly account for the very numerous amitoses since at the time of their breakdown there are, according to illustrations by Quisumbing and Juliano (1927), not more than a few hundred nucellar cells and each contains only a single nucleus.

An explanation of the curious chromosome-like structure of some of the nuclear fragments observed in the endosperm vesicles (Fig. 23, 24, 26, 40) may be found in changes in the tonicity of the suspending sol. Chromatin is highly sensitive to variations in tonicity and a change in the milk towards a hypertonic condition as a result of evaporation or condensation, with a corresponding increase in sugar content, might well account for the clumping and condensation of these nuclei and fragments. Such an increase in sugar content of the milk occurs at about the time these aberrations become most numerous. In fact the typical fixation image which is observed in the cells and nuclei of the sac at this time is characteristic of those which pertain when hypertonic conditions exist. Very similar nuclear effects have been noted in colchicine-treated plant cells by Levine (1951) and have been very beautifully demonstrated recently by Straus (1954) in endosperm of *Zea mays* grown *in vitro*.

While further study will be necessary to account for the specific basis of these various mitotic aberrations in *Cocos*, it is obvious that, during the period just prior to the precipitation of the free nuclei and vesicles in the liquid syncytium, radical changes are occurring in the cytoplasmic nuclear complex which are reflected by major disturbances in the usual pattern of nuclear behavior. Because of the ease with which these changes may be observed in *Cocos* and the components of the nuclear cytoplasmic system isolated, this tissue lends itself very well to experimental analysis.

SUMMARY

The occurrence of various nuclear aberrations and atypical nuclear divisions in the free nuclei, endosperm vesicles and endosperm meristem of *Cocos nucifera*

are reported. The formation of endosperm vesicles by the coalescence of cytoplasmic particles around free nuclei in the liquid syncytium of the developing embryo sac is described and figured. Several hypotheses are advanced to explain the apparent lack of mitosis during the development of the endosperm and it is suggested that this may result from hydrostatic pressure on, or changes in tonicity of the syncytial and cellular cytoplasm.

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A SPECIES OF *OLPIDIOPSIS* PARASITIC IN *ACHLYA GLOMERATA*

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Species concepts in the genus *Olpidiopsis* have recently been redefined by Shanor (1939a, b; 1940), McLarty (1939, 1941), Karling (1942), and Whiffen (1942). Despite these significant contributions to an understanding of species

variability and host ranges in the genus, identification of certain taxa remains difficult. The basis for this difficulty is apparently two-fold: First, the lack of thorough, concise species descriptions (particularly of type material) prior to 1939, and secondly, the frequently limited knowledge of host ranges. In an attempt to eliminate future uncertainties in the identification of species of *Olpidiopsis*, Karling (1942: 33) proposed four criteria which should be considered in taxonomic studies in *Olpidiopsis*. Satisfaction of Karling's criteria would insure a more certain identification of previously described species, and would serve as a basis for justifying the validity of new ones.

Among the water molds collected during a survey of the Saprolegniaceae in northern Mississippi, was a single specimen of *Achlya glomerata* which was heavily parasitized by a species of *Olpidiopsis*. Since *A. glomerata* has not previously been found to be subject to infection by *Olpidiopsis*, a study of the fungus, based on Karling's proposals, was initiated in an attempt to identify the parasite. The results of this investigation warrant the designation of the parasite as a new species. *Olpidiopsis fusiformis*, *O. saprolegniae* var. *saprolegniae*, *O. saprolegniae* var. *levis*, and *O. achlyae* were also collected, and compared with the fungus in *A. glomerata*.

Materials and Methods

The host, *Achlya glomerata*, was collected by the usual procedure of securing water molds, using hempseed halves as "bait". Monozoöspore isolation of the parasite and host was accomplished following the procedure outlined by Shanor (1939b: 181). The isolated two-member colonies were propagated in stock culture at 20°C. on halves of boiled, sterilized hempseed in 30 ml. of sterile, charcoal-filtered distilled water.

The method of cross-inoculation described by Shanor (1940) was utilized to determine the host range of the parasite. The influence of environment in establishing infection in water molds other than *Achlya glomerata* was studied by propagation of test hosts and infected colonies of *A. glomerata* (source of inoculum) in three types of water: sterile, charcoal-filtered, distilled water; sterile pond water; and unsterile pond water. Control cultures containing only colonies of the test hosts were propagated in the unsterilized pond water to serve as a check on the presence of other species of *Olpidiopsis*.

The influence of age of the host in securing infection was determined by subjecting 1, 3, and 7-day old test colonies to active cultures of *Olpidiopsis* in *Achlya glomerata*. Combinations of host age and type of culture water with incubation at 20° and 26°C. were employed to determine the effect of combined environmental variations on subsequent infection.

The possibility of variation in host strain susceptibility or resistance was considered. In the cross-inoculation studies, numerous test host isolates were used, each representing a separate collection or known strain of the host fungus. The number following the specific name represents the number of collections employed: *Achlya glomerata* (19, in addition to the original host specimen), *A. racemosa* (3), *A. colorata* (5), *A. flagellata* (17), *A. proliferoides* (5), *A. klebsiana*

(8), *A. diffusa* (10), *Saprolegnia delica-diclina*[1] (4), *S. ferax-mixta*[1] (8), *S. litoralis* (1), *Isoachlya subterranea* (5), *I. unispora* (7), *Dictyuchus anomalus* (3), *D. monosporus* (5), *Brevilegnia diclina* (1), *Geolegnia septisporangia* (1), *G. inflata* (2), *Pythium ultimum* (1), *P. rostratum* (1), *P. splendens* (1), *P. irregulare* (5), *P. arrhenomanes* (1), *P. debaryanum* (4), and *Aphanomyces laevis* (3). Each isolate was tested with the parasite 3 times in combinations of temperature, age, and type of culture water. No attempt was made to obtain monosporangial infection.

Description and Observations on a New Species

The parasite in *Achlya glomerata* commonly possesses roughened, minutely warted, or merely irregularly verrucose resting spores, hence the name *Olpidiopsis verrucosa* (figs. 1–24).

Olpidiopsis verrucosa n. sp.[2]

Zoösporangia terminal in the host hyphae, infrequently in short lateral branches; oval or spherical, very rarely pyramidal; 28 x 40 μ–84 x 103 μ, spherical ones 42–92 μ in diameter; smooth; commonly with one or two exit tubes. Zoöspores laterally biflagellate, isocont; oval or ellipsoid, rarely irregular; 3.6–4.8 μ in length by 2.4–3.0 μ in diameter. Resting spores (oögonia) oval, occasionally spherical, never ellipsoid; 22 x 26 μ–63 x 67 μ, predominantly 42–50 x 49–56 μ, inclusive of exospore. Exospore verrucose, crenulate, squarrose, or rugulose, infrequently irregular or bullate, rarely smooth, never echinulate; 1.4–8.4 μ thick. Companion cells (antheridia) 1–5 on a resting spore; spherical, occasionally ellipsoid or oval; smooth; 11–28 μ, predominantly 14–22 μ in diameter. Resting spore germinating by a single germ tube penetrating through the companion cell, the contents forming laterally biflagellate, isocont planonts.

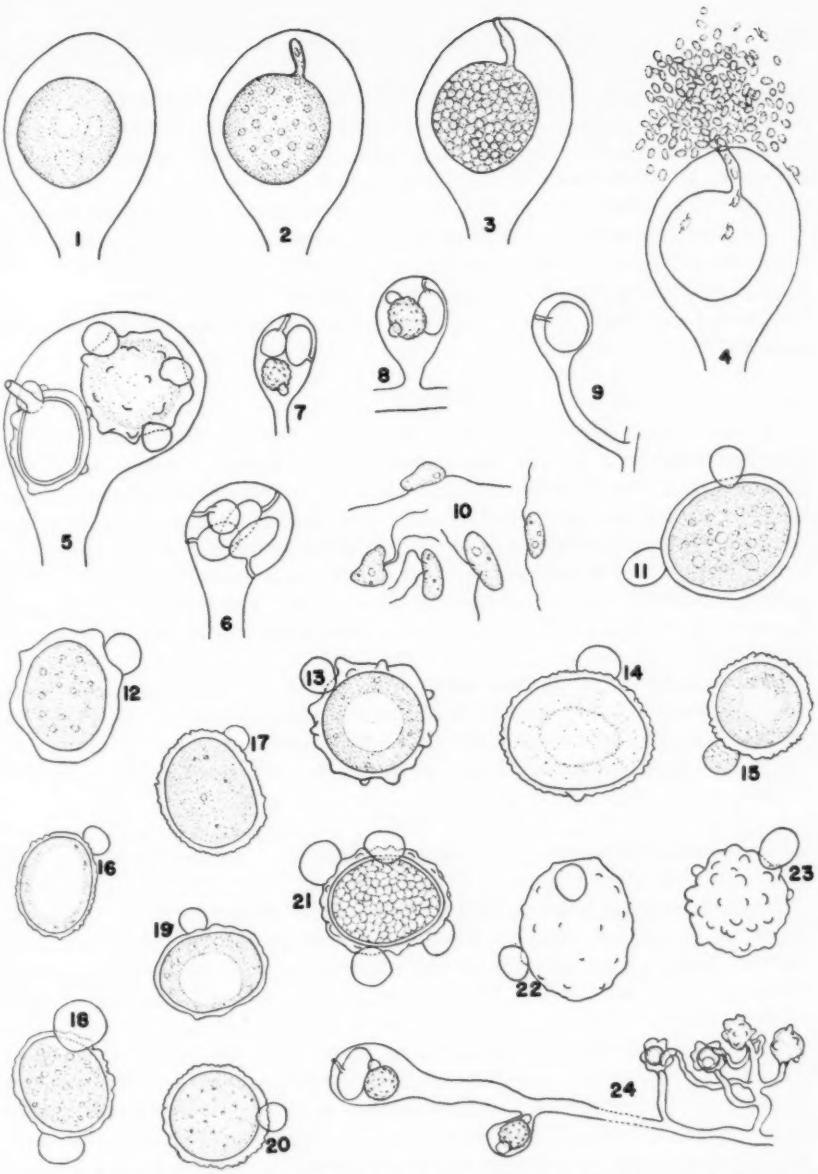
Parasitic in *Achlya glomerata*, from soil collected one mile east of Bagley Tower, Tallahatchie Experimental Forest, Lafayette County, Mississippi, September 20, 1953.

Zoosporangiis ovalibus vel sphaericis, infrequenter in pyramidis formam redactis; in magnitudine variis; in matrice terminalibus, raro in ramulis lateralibus habitantibus; quibus membrana levis. Zoosporis a latere biflagellatis, flagellis in longitudine paribus; 3.6–4.8 μ longis x 2.4–3.0 μ in diametro. Sporis quiescentibus (oogoniis) ovalibus vel interdum sphaericis, sed haud ellipsoideis, 22 x 26 μ usque ad 63 x 67 μ, plerumque 42–50 x 49–56 μ. Membrana exosporia verrucosa vel crenulata vel squarrosa vel rugulosa, raro irregulari vel bullata vel levi, haud echinulata. Cellulis consortibus (antheridiis) sphaericis vel interdum ellipsoideis vel ovalibus, cum membrana levi. In *Achlya glomerata* parasitans, ex solo lecta est in Tallahatchie Experimental Forest, Lafayette County, Mississippi, September 20, 1953.

Zoöspore discharge in *Olpidiopsis verrucosa* is worthy of a brief description. Immediately after leaving the zoösporangial exit tube the zoöspores assume a slight rocking motion, and shortly thereafter become quiescent. The zoöspores cluster around the orifice (Fig. 4) in a manner suggestive of the encysted spores in the genus *Achlya*. After 7–15 minutes of quiescence, the rocking motion is

[1] Until these taxa are studied in more detail so that species limits are more concisely defined, these collections cannot be more accurately identified.

[2] Latin description prepared by Dr. W. H. Willis, Department of Classics, University of Mississippi.



FIGS. 1–24. *Olpidiopsis verrucosa*. FIG. 1–4. Maturation of zoösporangium, and discharge of zoöspores. × 395. FIG. 5. Two resting spores, one in optical section after germination. × 395. FIG. 6. Discharged zoösporangia. × 90. FIG. 7. Zoösporangia and a resting spore in a host hyphal tip. × 90. FIGS. 8, 9. Infection in lateral branches of the host hyphae. × 90. FIG. 10. Zoöspores. × 870. FIGS. 11–21. Optical sections of resting spores, showing variations in exospore and companion cells. × 395. FIGS. 22, 23. Surface view of sparsely warted resting spores. × 395. FIG. 24. Portion of an older infected host hypha, and antheridia and oögonia of host. × 90.

again initiated, increases in rapidity, and within a few minutes the zoöspores dart away. Periodic addition of fresh water to the area immediately surrounding the zoösporangia prior to and during the discharge process, failed to alter the period of quiescent clustering of the zoöspores.

Butler (1907) described an achlyoid type of discharge and zoöspore behavior for *Olpidiopsis pythii*, and interpreted it as rudimentary diplanetism. The zoöspores of *O. verrucosa* do not leave cysts upon swimming away from the cluster, nor do the planonts "round up" during the interim quiescent period. It is doubtful, therefore, that the achlyoid zoöspore discharge is evidence of diplanetic behavior.

Host Range

Attempts to infect hosts other than *Achlya glomerata* with *Olpidiopsis verrucosa* were successful only in one case. One of 25 colonies of a fungus tentatively identified as *Achlya flagellata* was infected by *O. verrucosa*. Inoculation of this isolate of *A. flagellata* (?) with other species of *Olpidiopsis* failed to develop into infection. Of the 19 additional isolates of *A. glomerata* used in the cross-inoculation studies, 18 were susceptible to *O. verrucosa*. A single isolate of the host species appeared to be resistant to the parasite, even if exposed to zoöspores of *O. verrucosa* produced in 5 or more heavily parasitized colonies of the original host isolate.

Variations in age of the test hosts, temperature of incubation, and type of culture water were not influential in bringing about infection. Reciprocal infection, however, was consistently obtained between parasite-free colonies of the original host isolate and all other "susceptible" isolates of *A. glomerata*.

Discussion

Olpidiopsis verrucosa is easily distinguishable from all previously described species in this genus except *O. incrassata* Cornu (1872), *O. major* Maurizio (1895), and *O. vexans* Barrett (1912). There is some question as to the validity of the two latter species; Sparrow (1943) considered them to be doubtfully valid, whereas Karling (1942), with reservation, reduced them to synonymy under Cornu's *O. incrassata*. These three species are considered here to be distinct entities merely for purposes of comparison with *O. verrucosa*.

Olpidiopsis incrassata is characterized by a markedly undulant exospore (Cornu, 1872: pl. 4, fig. 12). Only one of the resting spores observed in *O. verrucosa* (Fig. 12) was even remotely suggestive of those of *O. incrassata*. In this characteristic at least, *O. verrucosa* is quite distinct from Cornu's species. *Olpidiopsis major* and *O. verrucosa* are also separable on the same morphological characteristic.

Olpidiopsis saprolegniae Fischer is not, according to Barrett (1912: 231), the same as Cornu's *O. saprolegniae*. Consequently, Barrett raised Fischer's species to specific rank under the epithet *O. vexans*. Illustrations of *O. vexans* Barrett (*O. saprolegniae* Fischer) in the publication by Fischer (1882) show the exospore to be densely warted or "knobby". Such resting spores are not produced by *O.*

verrucosa, although sparsely-warted ones were (rarely) observed (Figs. 13, 23). Barrett illustrated the exospore of *O. vexans* with two figures (1912: pl. 24, fig. 27; pl. 25, fig. 47). Only figure 27 is suggestive of *O. verrucosa* (see fig. 15). According to Karling, the exospore of *O. vexans* is often echinulate. None of the resting spores of *O. verrucosa*, however, were observed to be echinulate. Resting spore and zoösporangial sizes in *O. verrucosa* and *O. vexans* are similar. The fundamental morphological difference between these two species, therefore, is the presence of echinulate zoösporangia and resting spores in the latter, and the lack of these in the former.

Olpidiopsis incrassata, *O. major*, *O. vexans*, and *O. verrucosa* cannot be separated solely on a morphological basis. Host ranges in these species, however, are markedly dissimilar, and coupled with structural differences, form the basis for considering *O. verrucosa* to be a distinct species. *Olpidiopsis incrassata* reportedly parasitizes only species of *Saprolegnia* (Sparrow, 1943), and two species of *Isoachlya* (Shanor, 1940); *O. major* is parasitic only in species of *Saprolegnia*, and *O. vexans* parasitizes *Saprolegnia* and unidentified species of *Achlya* (Sparrow, 1933; Petersen, 1910). *Olpidiopsis verrucosa* is presently known to be confined to *A. glomerata*, and only doubtfully can the range be extended to *A. flagellata*. Neither *A. glomerata* nor *A. flagellata* has previously been reported as host for species of *Olpidiopsis*.

Olpidiopsis vexans and *O. verrucosa* are, admittedly, closely allied species, but it is believed they can be recognized as separate entities, unless future investigations bring to light new and conclusive evidence to the contrary, on the basis of host range and morphology. It is, of course, entirely possible that *O. verrucosa* is only a biological race of *O. vexans*, or even of *O. incrassata*. If such were the case, however, a more inclusive host range for *O. verrucosa* would be expected. Failure to infect even one species of *Saprolegnia*, and a very limited host range in the genus *Achlya*, certainly implies that *O. verrucosa* is not a race of *O. vexans*.

The results of the experimental work on host range, and the morphological observations on *Olpidiopsis verrucosa* seem to have satisfied the criteria suggested by Karling. The species should stand as a distinct taxon, at least until a more suitable means of species differentiation in the genus is formulated.

Summary

Olpidiopsis verrucosa is described as a new, holocarpic, endobiotic species, parasitic in *Achlya gomerata*. The characteristics of *O. verrucosa* suggest *O. vexans* and *O. incrassata*, but it differs from these species primarily in configuration of the exospore, and in host range. *Olpidiopsis verrucosa* is limited in host range to *Achlya glomerata*, and is only doubtfully parasitic in an aberrant strain of *A. flagellata*(?).

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considerable task of propagation and inoculation of test host cultures, and for the collection and isolation of these fungi.

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AN EMBRYOLOGICAL STUDY OF FOUR SPECIES OF ASARUM*

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Of the six genera of the Aristolochiaceae (*Asarum* L., *Aristolochia* Tourn., *Holostylis* Duch., *Saruma* Oliv., *Bragantia* Lour., and *Thottea* Rottb.) only two, *Asarum* and *Aristolochia*, have been investigated embryologically (Dahlgren,

* This paper was condensed from a thesis presented to the faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Botany.

1915; Hegelmaier, 1886; Hofmeister, 1858, 1859; Jacobsson-Stiasny, 1918; Kratzer, 1918; Leemann, 1927; Samuelsson, 1914; Tackholm and Soderberg, 1918; Warming, 1878). Kratzer's account of *Aristolochia clematitis* L. and *Asarum europaeum* L. (1918) is the most complete, but it is lacking in details of embryo sac formation, fertilization, endosperm formation, early embryo development, and seed development.

In the present study, a detailed embryological investigation has been made with *Asarum arifolium* Michx., *A. virginicum* L., *A. Lewisii* Fern., and *A. canadense* L. var. *reflexum* (Bickn.) Robins., all growing in the vicinity of Chapel Hill, North Carolina. These species represent two of the four sections as defined by Solereder (1889). *Asarum arifolium*, *A. virginicum*, and *A. Lewisii* belong to the section Ceratasarum, while *A. canadense* is a member of the section Euasarum. The other two sections, Heterotropa and Ascidasarum, are represented by species occurring in Japan only.

Microsporogenesis and microgametogenesis were not studied in any of the species. As far as is known by the author, nothing has been published on the embryology of either of these species of *Asarum*. The results of the investigation are in agreement with those reported by other workers for *Asarum europaeum*.

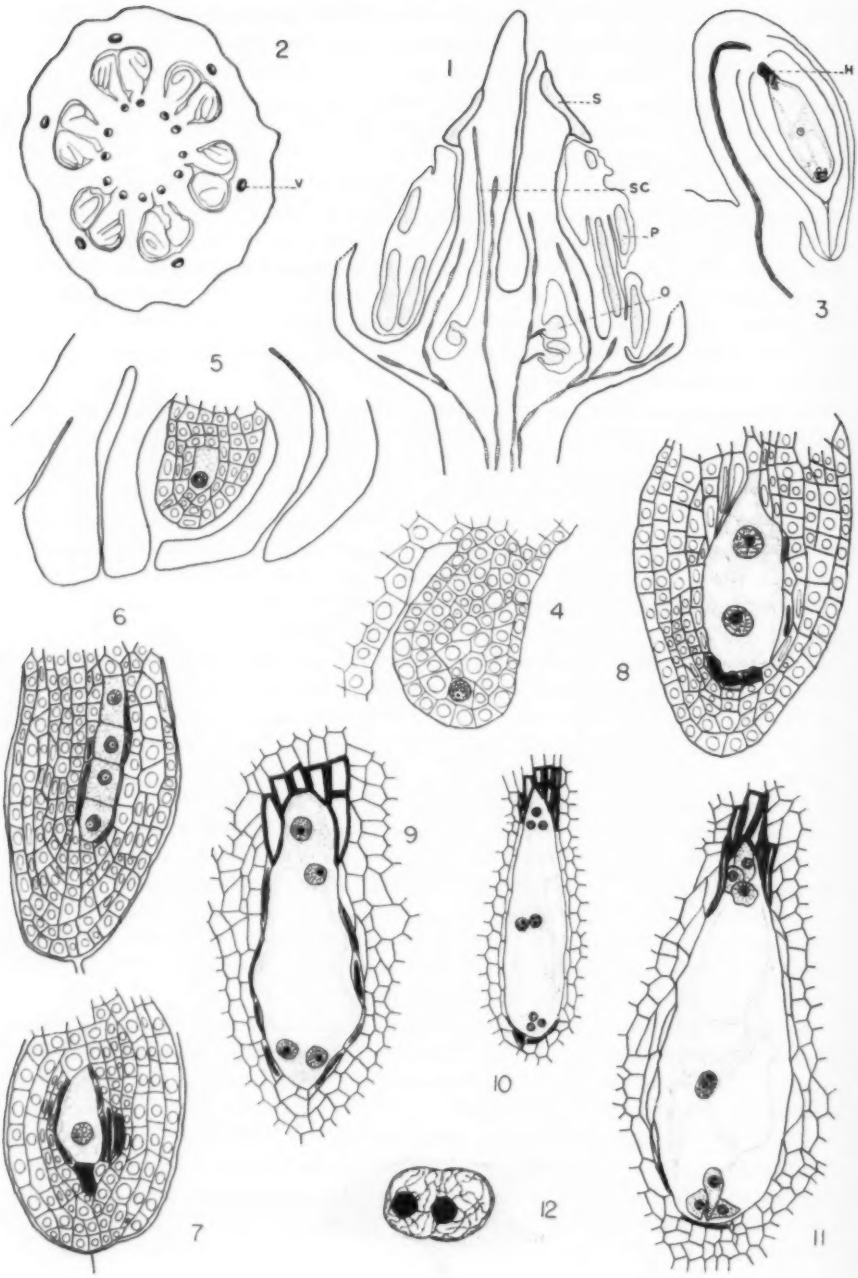
Buds, open flowers, and fruits of *Asarum arifolium* and *A. virginicum* were collected at weekly intervals from January 23, 1953 to May 14, 1953, at Meeting-of-the-Waters, Chapel Hill. These were supplemented by collections of *A. arifolium* from Meeting-of-the-Waters on October 17, 1952, November 13, 1953, and December 12, 1953. Buds in all stages, open flowers, and fruits of *Asarum canadense* were collected at Deep River, Lee County, North Carolina, on March 27 and April 11. Buds, open flowers, and fruits of *Asarum Lewisii* were collected in Wake County, North Carolina, on April 13 and June 3. Plants from each of the above populations were brought into the greenhouse and potted for further study.

The material collected from the vicinity of Chapel Hill was fixed in Randolph's modified Navashin Fluid and Formalin-Aceto-Alcohol. Those collected elsewhere were fixed in either Formalin-Aceto-Alcohol or Formalin-Propiono-Alcohol. Randolph's modified Navashin Fluid seemed to give the best results.

Dehydration was by the tertiary butyl alcohol method (Johanssen, 1940) and the material was embedded in paraffin in the usual way. Whenever practical, the entire bud or flower was embedded for sectioning, but usually the flowers and fruits were dissected and only the ovary embedded.

Sections were cut at ten, twelve, thirteen, fourteen, and fifteen microns, depending upon the age of the material, the thicker sections being used for older stages. These were stained in Heidenhain's iron-alum haematoxylin without a counter stain and mounted in balsam.

For gross morphological study, flowers of each of the four species studied were treated with a dilute solution of sodium hydroxide for one week at 60°C., washed in boiling water, dehydrated with ethyl alcohol, and cleared in toluene. The material was well suited to this type of treatment, and the vascular supply of the flower or fruit could be studied easily.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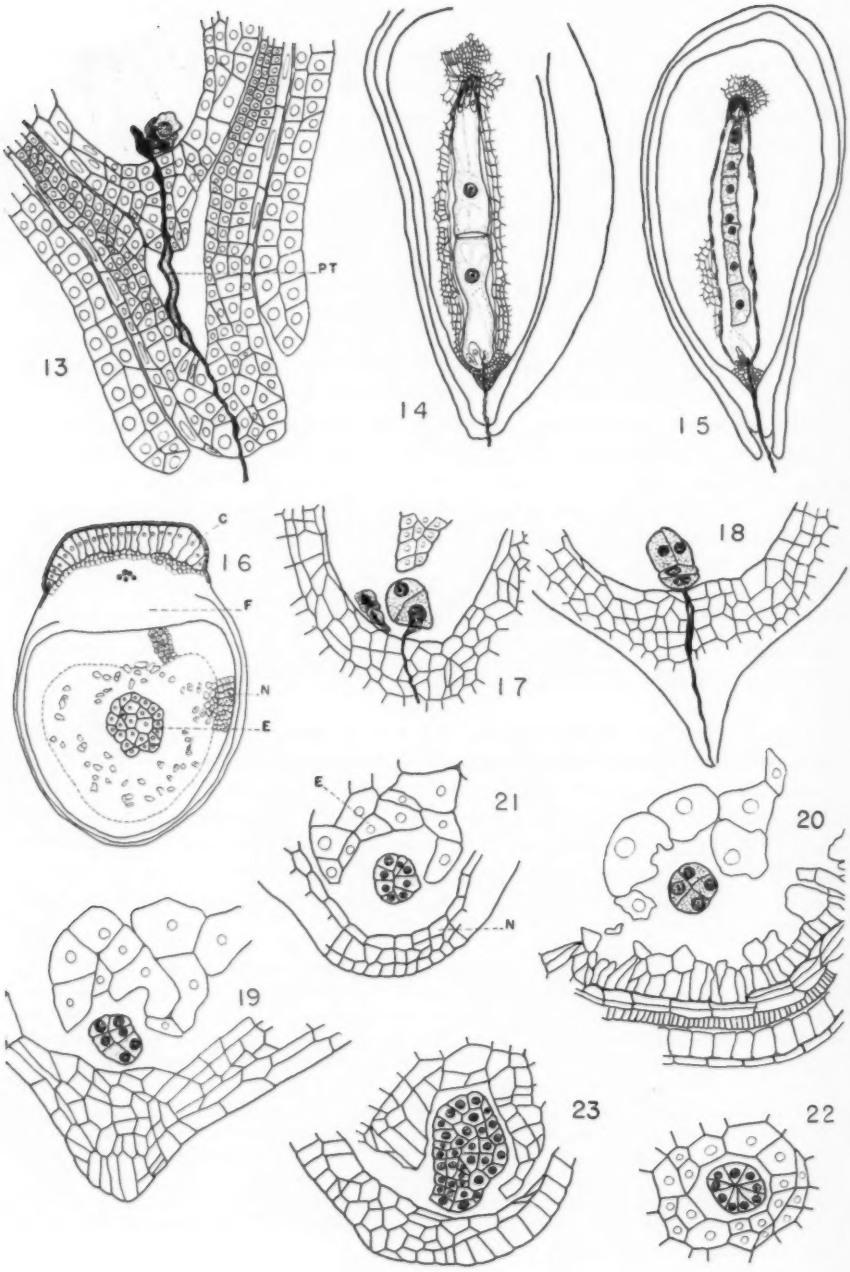
ASARUM ARIFOLIUM

OVARY AND OVULE. –The ovary of *Asarum arifolium* is half-inferior, the calyx tube being adnate only to the lower half of the ovary (Fig. 1). The pistil is composed of six carpels united at their bases but free above to form a "stylar" portion which ends in a pair of horns. Each locule contains five to eight anatropous ovules in two longitudinal rows and extends into the "stylar" portion of the carpel to the stigma to form a "stylar canal" (Fig. 1). The stigmas are small, about one mm. in diameter, principally extrorse, but often with a small introrse stigmatic surface below the horns (Fig. 1). Each carpel is supplied by three main vascular bundles: one dorsal, which is double, and two ventral (Fig. 2). The two ventral strands send out traces to the two rows of ovules. Vascular tissue is present only in the funiculus of the ovule and does not extend into either the integuments or nucellus (Fig. 3). The dorsal strand divides at the tip and anastomoses with the tips of the two ventral strands.

The ovule is bitegumentary, the outer integument of two cell layers, the inner of three, except around the micropyle where it may become four- or five-layered (Fig. 13). The integuments begin their development early and are easily distinguished at the megaspore mother cell stage (Fig. 5). The micropyle is formed in most instances by both the outer and inner integuments, the outer integument overlapping the inner integument at the micropylar end (Fig. 3). At first all the cells, with the exception of the apical cells at the micropylar end of the ovule, are cuboidal with a large nucleus. The apical cells are triangular, indicating anticlinal divisions (Fig. 13). During the formation of the seed, the integuments undergo major alterations which will be discussed in connection with the mature seed.

The nucellus is broad and massive, and mitotic figures can be found toward its periphery through the time of early endosperm formation (Fig. 3). The nucellar epidermis contains denser cytoplasm than the remaining cells of the nucellus. This layer at first is made up of cuboidal cells which elongate during seed formation. There is a gradual disappearance of the nucellus during seed formation, and by the time the seed is shed, the only cells of the nucellus remaining are those of the nucellar epidermis and a small group of cells at the micropylar and chalazal ends (Figs. 24, 25).

FIGS. 1–12. *Asarum arifolium*. FIG. 1. Longitudinal section of a young flower. × 19. *S*, stigma; *SC*, stylar canal; *P*, pollen sac; *O*, ovule. FIG. 2. Cross section through the ovary of a young flower. × 19. *V*, vascular bundle. FIG. 3. Longitudinal section of an ovule just before fertilization. × 44. *H*, hypostase. FIG. 4. Longitudinal section of a very young ovule showing the archesporial cell. × 300. FIG. 5. Longitudinal section of a young ovule showing integuments and megaspore mother cell. × 300. FIG. 6. Longitudinal section of nucellus with tetrad of megaspores. × 300. FIG. 7. Longitudinal section of nucellus with functional megaspore and remains of the other three megaspores. × 300. FIG. 8. Longitudinal section of nucellus and two-nucleate embryo sac. × 300. FIG. 9. Longitudinal section of four-nucleate embryo sac, showing hypostase and large central vacuole. × 300. FIG. 10. Longitudinal section of polarized eight-nucleate embryo sac. × 181. FIG. 11. Longitudinal section of embryo sac with the two polar nuclei fusing. × 181. FIG. 12. Enlarged view of the two polar nuclei fusing prior to fertilization. × 670.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The integuments originate near the base of the nucellus (Fig. 3). At the level of their origin there is a well-defined, irregularly outlined group of nucellar cells, elongated longitudinally and poor in cytoplasmic contents but with thick, yellowish (suberized?) walls of a highly refractive character (Fig. 11). This group of cells, termed the "hypostase" by Van Tieghem (1901), forms a cap over the chalazal end of the embryo sac, with the antipodal cells at first being encased by the cap. The cells immediately above the hypostase are filled with a yellowish substance. At the micropylar end the cells of the nucellar epidermis form a beak or plug extending into a cavity formed by the integuments (Fig. 13). This plug is persistent throughout seed formation.

THE FEMALE GAMETOPHYTE.—The archesporium is one-celled and of hypodermal origin (Fig. 4). It can be seen quite early, even before the appearance of the integuments, distinguishable from the surrounding cells by its denser cytoplasm and larger nucleus. Ovules collected on October 17, 1952, were in the archesporial stage. The archesporial cell divides to form a primary parietal cell and a primary sporogenous cell. The primary parietal cell divides to form several wall layers and eventually the primary sporogenous cell is pushed back into the nucellar tissue (Fig. 5). The primary sporogenous cell functions as the megaspore mother-cell. The usual meiotic divisions form a tetrad of four cells in a vertical row (Fig. 6). The megaspore mother-cell was observed in ovules collected on November 13, 1953, and the tetrad of spores in ovules collected on December 12, 1953. Of the tetrad of spores, the three nearer the micropyle degenerate but persist as faintly outlined cells with dark-staining, irregular-shaped nuclei (Figs. 7 and 8). The remaining megaspore becomes the functional megaspore.

The development of the embryo sac follows the monosporic eight-nucleate *Polygonum* (normal) type (Maheshwari, 1950). The functional megaspore just before division is approximately 46 microns long and 23 microns in diameter and lies in the center of the nucellar tissue (Fig. 7). Increase in size of the megaspore is accompanied by vacuolation. The megaspore nucleus divides to produce two nuclei which move to opposite poles (Fig. 8). Vacuolation continues, and at the two-nucleate stage two or three large vacuoles lie in the center of the sac separating the two nuclei. At this two-nucleate stage the sac measures about 130 microns in length. A four-nucleate stage follows the two-nucleate stage, (Fig. 9), with a concomitant increase in length of the sac to about 200 microns and the

FIGS. 13–23. *Asarum arifolium*. FIG. 13. Longitudinal section of the micropylar end of an ovule at the time of fertilization. × 181. *PT*, pollen tube. FIG. 14. Longitudinal section of ovule with two-celled endosperm. × 44. FIG. 15. Longitudinal section of ovule with eight-celled endosperm. × 44. FIG. 16. Cross section of ovule with early endosperm. × 44. *C*, caruncle; *F*, funiculus; *N*, nucellus; *E*, endosperm. FIG. 17. Longitudinal section of two-celled embryo with portion of endosperm, nucellus, pollen tube, and the two degenerate synergids. × 181. FIG. 18. Longitudinal section of four-celled embryo. × 181. FIG. 19, Longitudinal section of six-celled embryo. × 181. FIG. 20. Cross section through twelve-celled embryo. × 181. FIG. 21. Longitudinal section of sixteen-celled embryo. × 181. *E*. endosperm; *N*, nucellus. FIG. 22. Cross section through twenty-four-celled embryo. × 181. FIG. 23. Longitudinal section of embryo at time seed is shed. × 181.

formation of a single, large, central vacuole. The cytoplasm forms a thin peripheral layer around the sac with aggregations of cytoplasm around the two sets of nuclei. This four-nucleate stage is followed by an eight-nucleate stage with four cells congregated at each end of the sac. Two polar nuclei, one from each pole, migrate to the center of the sac (Fig. 10).

The mature embryo sac is about 450 microns long (Fig. 11). It is slightly larger at the micropylar than at the chalazal end and is circular in cross section. The micropylar end is quite rounded, but the chalazal end is drawn out into a cone-shaped form which fits up into a similarly shaped mold formed by the hypostase. The mature embryo sac consists of an egg and two synergids at the micropylar end of the sac, a secondary nucleus formed from the fusion of the two polar nuclei in the center of the sac, and three antipodal cells at the chalazal end.

The egg apparatus (Fig. 11) is composed of an egg and two synergids which are extremely difficult to fix and stain properly. The two synergids are triangular in shape as seen in longitudinal section, each with a dark-staining nucleus about 10 microns in diameter. The synergids begin to degenerate very soon after they are formed, and at the time of fertilization they persist only as dark-staining bodies. The egg is more or less club-shaped, with a nucleus about 10 microns in diameter in the lower part of the cell. A large vacuole can be seen, in most cases, in the upper part of the cell.

The two polar nuclei before fusion are approximately the same size (Fig. 10), measuring about 13 microns in diameter. They usually occupy a position approximately in the center of the sac, but in some cases can be found in the upper third. Fusion of the polar nuclei to form a secondary nucleus occurs immediately after their migration to the center of the sac and quite some time before the pollen tube enters the sac (Figs. 11, 12). The secondary nucleus thus formed measures about 23 microns in diameter at the time of fertilization. Thin protoplasmic strands connect it with the egg apparatus.

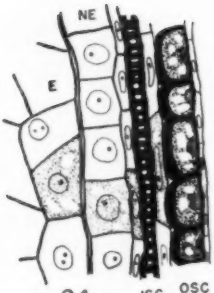
The antipodals (Fig. 11) when first formed are surrounded by the cells of the hypostase. The nuclei at this time measure about 10 microns in diameter. As the embryo sac enlarges, the antipodals enlarge also and begin to extend down farther into the sac. At the time that the polar nuclei fuse, the nuclei of the antipodal cells have increased to about 16 microns, and vary in shape from spherical to oblong. They contain deeply-staining chromosomes. The antipodals persist until the embryo sac has become almost filled with endosperm, and their nuclei attain a diameter of about 30 microns. The rapid increase in size of the antipodal nuclei and the presence, after their formation, of distinct chromosomes, suggests polyteny. In no case was either mitosis or cytokinesis observed.

FERTILIZATION.—Microsporogenesis and microgametogenesis were not observed in *Asarum arifolium*. Microsporogenesis occurred prior to October 17, in 1952, and on January 23, 1953, the pollen was in the two-celled stage. The pollen is shed early in March as the flowers begin to open. Pollination follows very soon thereafter, with fertilization occurring immediately after the sperms are discharged from the pollen tube.

The pollen tube must travel a distance of only two to six millimeters before reaching the ovule, since the "styles" in this case are very short. The "style" of *Asarum arifolium*, as previously described, is of the "open" type, with a wide "stylar canal" through which the pollen tube travels on its way to the ovule (Fig. 1). The inner epidermis of the stylar canal appears to assume the function of nutrition and conduction of the pollen tube. After entering the micropyle the tube passes through an open chamber above the nucellus, then makes its way through the "nucellar beak" to the embryo sac (Fig. 13). It penetrates the sac only a short distance before it discharges its sperms. One of the sperms unites with the egg and the other apparently makes its way to the secondary nucleus in the center of the sac, where it fuses with it. The migration and fusion of this sperm with the secondary nucleus were not observed, however. Actual union of egg and sperm was not observed, but a dark-staining, rod-shaped body which could be interpreted only as a sperm was seen lying in the egg cell close to the egg nucleus (Fig. 13). Fusion of the sperm with the egg apparently takes place while the egg is in the resting stage. The pollen tube collapses soon after fertilization, but persists as a yellow-staining structure during early embryonic development (Fig. 15).

THE ENDOSPERM.—As has already been stated, fusion of the two polar nuclei occurs some time prior to fertilization (Fig. 11). Immediately after fusion of the polar nuclei, two nucleoli are evident. By the time of fertilization, however, there is only one large nucleolus. Fusion of the secondary polar nucleus with the sperm occurs soon after the sperm has been released from the pollen tube, as evidenced by the early division of the endosperm mother nucleus. The first division of the endosperm mother nucleus is transverse. Immediately after division, a cross wall is laid down which divides the sac into two chambers (Fig. 14). The second and third divisions are likewise transverse and are followed by the laying down of cross walls. This results in a row of eight cells extending from the micropylar to the chalazal end of the sac (Fig. 15). The type of development just described is in agreement with that described by Maheshwari (1950) for other genera of the Aristolochiaceae. From the eight-celled endosperm stage, both vertical and transverse divisions occur (Fig. 16) until the embryo sac is completely filled with endosperm cells and all the nucellar tissue, except the nucellar epidermis and a group of cells at the chalazal and micropylar ends of the sac, has disappeared. All divisions are followed by wall formation. Thus, endosperm formation is of the cellular type. The divisions are synchronous through the eight-celled stage, but after this stage nuclei can be found in all stages of division. The endosperm is persistent in the mature seed. At the time that the seed is shed, only a very small fraction of the endosperm immediately surrounding the embryo has disappeared. The greatest bulk of the mature seed is endosperm (Fig. 25).

THE EMBRYO.—The first division of the zygote is delayed until the endosperm is at the twelve- to sixteen-celled stage. This first division is transverse, cutting



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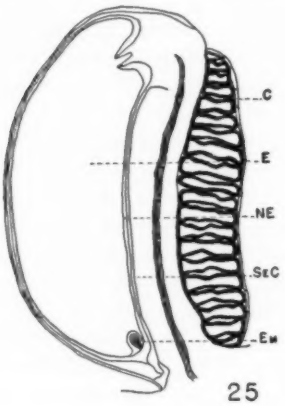

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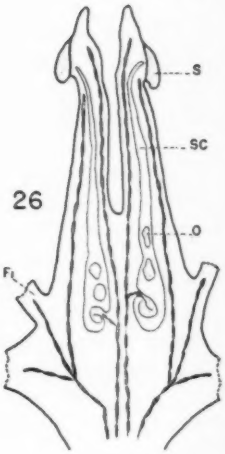

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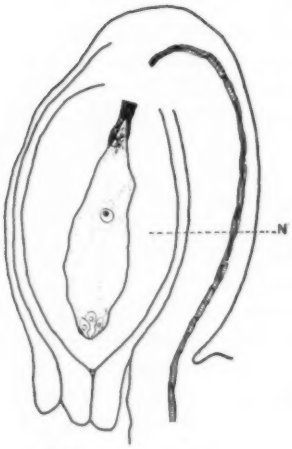

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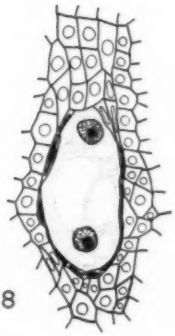
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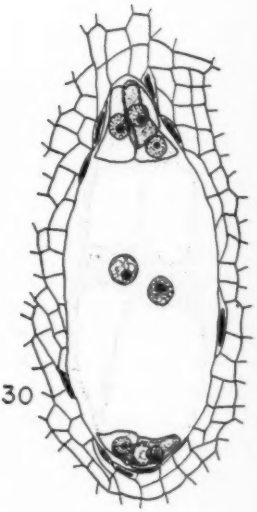
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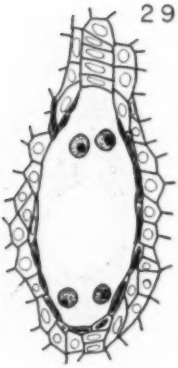
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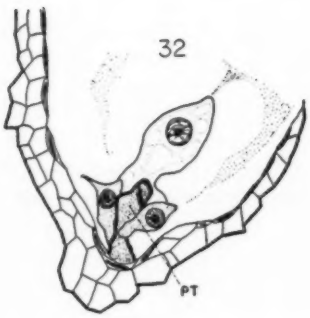

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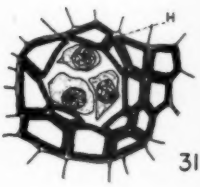

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off a terminal and a basal cell (Fig. 17). The terminal cell then divides vertically while the basal cell divides transversely (Fig. 18). This is followed by a vertical division of each of the two cells formed from division of the basal cell and results in three two-celled tiers of cells (Fig. 19). Several vertical, radial divisions occur in each tier, producing a sphere of twenty to twenty-four cells (Figs. 20–22). More transverse and several vertical divisions occur, so that when the seed is mature the embryo consists of a spherical mass of undifferentiated cells lacking a well differentiated suspensor, cotyledons, hypocotyl or epicotyl (Fig. 23).

THE MATURE SEED.—The seeds of *Asarum arifolium* were shed in April and May of 1953. When the seeds are shed they are about 3 mm. long, firm, and narrowly ovate. The funiculus is still intact and rather massive in the seed after it is shed, and there is a prominent caruncle running the entire length of the funiculus (Fig. 25). The caruncle originates from the second cell layer of the funiculus. The cells are very large, comparatively thick-walled, contain a large central vacuole, and usually are collapsed in the mature seed. Mucilage is produced by the cells, giving the funiculus a sticky appearance.

The seed coat is made up of five layers of cells (Fig. 24), the first two of which are derived from the outer integument and the other three from the inner integument. These five are as follows: An outer layer of thin-walled, greatly elongated, narrow cells which disappear soon after the seed is shed; a layer of cubical cells with the inner wall greatly thickened and the nucleus pressed against the outer wall; a layer of elongated, narrow, relatively thin-walled cells; a layer of small cubical sclerenchyma cells; and an inner layer of elongated dark-staining cells, thin-walled, containing starch.

The epidermis of the nucellus is persistent immediately under the seed coat. It consists of a single layer of large, thin-walled, rectangular cells (Fig. 24).

The endosperm is fleshy and fills most of the seed, bounded by the nucellar epidermis (Fig. 25). The cells are compact, small, and rectangular or cubical (Fig. 24). The nuclei are relatively large and lie embedded in dense cytoplasm. Most of the nuclei contain two nucleoli, but as many as seven were counted in some cells.

The embryo is a spherical mass of undifferentiated cells with a very minute suspensor (Fig. 25). The cells are small and each contains a large nucleus in dense

FIGS. 24, 25. *Asarum arifolium*. FIG. 24. Longitudinal section through wall of mature seed. × 300. *E*, endosperm; *NE*, nucellar epidermis; *ISC*, inner seed coat; *OSC*, outer seed coat. FIG. 25. Longitudinal section of a mature seed. × 19. *C*, caruncle; *E*, endosperm; *NE*, nucellar epidermis; *SeC*, seed coat; *Em*, embryo.

FIGS. 26–32. *Asarum virginicum*. FIG. 26. Longitudinal section of a young flower. × 19. *Fi*, filament; *S*, stigma; *SC*, stylar canal; *O*, ovule. FIG. 27. Longitudinal section of ovule with mature embryo sac. × 69. *N*, nucellus. FIG. 28. Longitudinal section of two-nucleate embryo sac. × 300. FIG. 29. Longitudinal section of four-nucleate embryo sac. × 300. FIG. 30. Longitudinal section of polarized eight-nucleate embryo sac. × 300. FIG. 31. Cross section through hypostase showing antipodal cells during early endosperm formation. × 300. *H*, hypostase. FIG. 32. Longitudinal section of the egg apparatus at the time of fertilization. × 300. *PT*, pollen tube.

cytoplasm. Endosperm completely surrounds the embryo except at the micropylar end.

The hypostase is still present and prominent in the mature seed. The antipodals, however, have disappeared by this time and there is no evidence of their presence (Fig. 25).

The vascular supply to the seed consists entirely of spiral vessels. The single strand extends almost to the hypostase, but does not enter the seed coat or the endosperm (Fig. 25).

Asarum virginicum

The development of the ovule to the mature seed in *Asarum virginicum* parallels that of *Asarum arifolium*, with a few minor exceptions.

Asarum virginicum has an ovary which is only very slightly inferior, being fused with the calyx tube only at its base (Fig. 26). The pistil consists of six carpels united at the bottom but free at the top to form a "stylar" portion which is about 2 mm. long and ends in a pair of horns which are fused except at their tips. An extrorse stigmatic surface is present below the horns, but there is no introrse stigmatic surface such as is sometimes present in *Asarum arifolium*. Each locule contains from six to eight anatropous ovules and extends into the "stylar" portion of the carpel to the stigma to form a "stylar canal" (Fig. 26).

The ovule (Fig. 27) is similar in structure to that of *Asarum arifolium*. The micropyle, however, is always formed from the inner integument only and the hypostase is more prominent.

Megasporogenesis in this species follows the same pattern as that of *A. arifolium*, as does the development of the embryo sac. The embryo sac increases gradually in size from about 100 microns at the two-nucleate stage (Fig. 28) to about 170 microns at the four-nucleate stage (Fig. 29), to about 560 x 140 microns at the seven-nucleate stage. At the eight-nucleate stage all of the nuclei are approximately ten microns in diameter (Fig. 30). The egg (Fig. 32) increases to about 17 microns after fertilization, and the first division of the zygote occurs somewhat later than in *A. arifolium*. In contrast to *A. arifolium*, the synergids are persistent and metabolically active until after fertilization (Fig. 32), when they begin to degenerate and stain very darkly.

The secondary nucleus formed by the two polar nuclei is about 33 microns in diameter. The development of this cell into the mature endosperm follows the same general pattern as in *A. arifolium*. The antipodals gradually increase in size from ten microns when they are first formed to about 23 microns when the embryo is at the eight-celled stage. They persist until the embryo sac has been almost filled with endosperm, at which time they disappear. At no time do they extend very far down into the sac, for the most part remaining in and surrounded by the hypostase. Chromosomes are evident as in *A. arifolium* and appear to be composed of numerous strands (Fig. 31). Again polyteny is suggested.

One of the flowers investigated showed a majority of its ovules with an aberrant type of embryo sac development. Some showed as many as thirty-two nuclei arranged peripherally around the sac, with a large central vacuole (Fig. 33). There was no distinction between egg, synergids, polar nuclei, or antipodals, and

no cross walls were present. A later stage showed these thirty-two nuclei arranged in three groups, a micropylar group, a chalazal group, and a central polar group (Fig. 34). The central nuclei were in the process of fusing, and as many as sixteen nuclei were observed to take part in the process. It could not be determined whether or not this embryo sac was functional.

The mature seed (Figs. 35, 36) is similar to that of *A. arifolium* in structure. The embryo, however, is not as large as in *A. arifolium* when the seed is shed.

Asarum Lewisii

The development of the seed of *Asarum Lewisii* is very similar to that of *Asarum arifolium*, but it more nearly parallels that of *A. virginicum*.

The ovary is completely superior, in contrast to that of both *A. virginicum*, which is only slightly inferior, and *A. arifolium*, which is half-inferior. The "stylar" portion of the carpel is about 3 mm. long and terminates with a pair of fused horns about 1 mm. long. Only an extrorse stigmatic surface is present. The locule, which contains up to twelve ovules, extends into the "stylar" portion to the stigma to form a "stylar canal", as in the other two species.

The embryo sac increases gradually in size to a maximum of 322 x 84 microns at the seven-nucleate stage (Fig. 38). This is the smallest embryo sac of the four species.

The antipodals persist for a long time, as in *A. virginicum*, but do not greatly enlarge. They do not extend into the embryo sac, but remain encased by the hypostase. The first division of the zygote occurs at about the same stage of endosperm formation as in *A. virginicum*. The synergids are persistent at fertilization, after which they disappear.

As in *A. virginicum*, the micropyle is formed from the inner integument only (Fig. 37). The nucellus separates from the integument at the micropylar end to form a prominent cavity (Fig. 37). Such a cavity was observed in *A. arifolium* (Fig. 13), but to a lesser extent. *Asarum virginicum* (Fig. 27) and *A. canadense*, (Fig. 40) showed much smaller cavities.

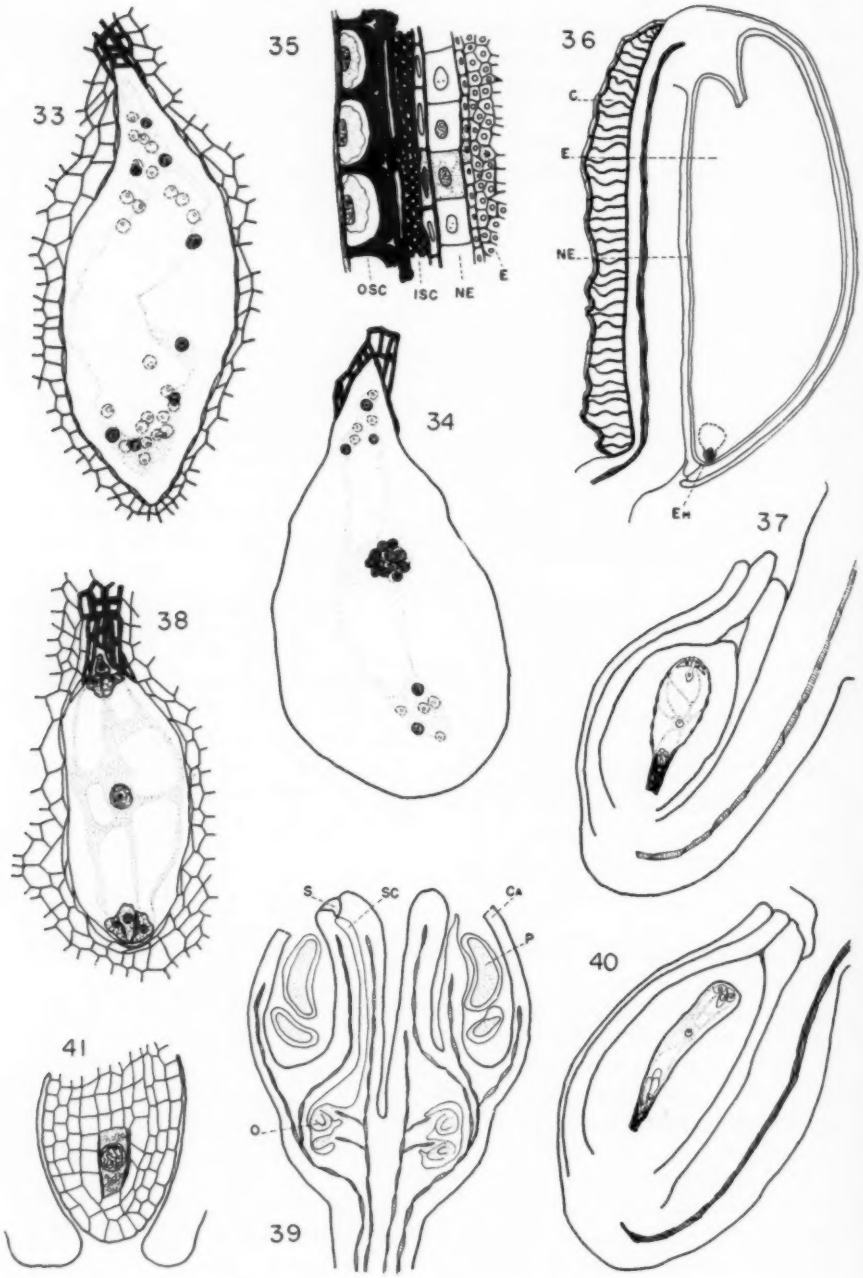
Asarum canadense

The seed development of *Asarum canadense* parallels that of the other three species studied.

The ovary in this case is completely inferior (Fig. 39). The six styles, about 3 mm. long, are fused except at their tips, where each terminates in a pair of free horns. The stigmatic surface is extrorse, more terminal than in any of the other three species, and extends between the horns. The locule, which contains about eight ovules, extends up into the style to the stigma to form a "stylar canal" as in the other species (Fig. 39). The vascular supply to the carpel is similar to that of the other three species.

The structure of the ovule is similar to that of *Asarum arifolium*. The only basic difference is that the micropyle is formed from the inner integument only and is more prominent (Fig. 40).

Megasporogenesis (Figs. 41–43) follows that of the other species of *Asarum*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with one exception. In *Asarum canadense* it is the micropylar member of the megaspore tetrad which becomes the functional megaspore (Figs. 42, 43).

The embryo sac increases gradually to a maximum size, at the seven-nucleate stage, of about 500 x 125 microns (Figs. 44–46). In the mature embryo sac (Fig. 46), the polar nuclei lie toward the micropylar end instead of in the center of the sac, and the antipodals, which contain a large vacuole toward their micropylar ends, extend about one-half to three-fourths of the way into the sac. The outstanding difference in seed development between this and the other species is the tremendous increase in size of the antipodal nuclei and cells after their formation. The antipodal nuclei are about 10 microns in diameter when they first become polarized (Fig. 47). At this stage the chromosomes are so long and drawn out that they appear as the typical "chromatin network" after fixation. By the time that the polar nuclei have fused, the antipodal nuclei have increased to a size of 23 microns, with no chromosomes, as such, yet visible (Fig. 48). Just before fertilization they attain a size of 33 microns, and at this stage chromosomes of at least two or three chromonemata are clearly visible (Fig. 49). The nuclei continue to increase in size until at the sixty-four-celled endosperm stage they reach their maximum diameter of about 66 microns (Fig. 51). At this stage, giant chromsomes composed of numerous chromonemata are outstanding. On those chromosomes not stained too deeply, bands of chromomeres could be easily mapped (Fig. 52). The nuclei at their maximum size occurred in several sections so that it was impossible to count the chromosomes. The structure of the chromosomes and the phenomenal increase in size of the nuclei very strongly suggest polyteny of the sort found in the salivary glands of *Drosophila* and other Diptera. Apparently there have been repeated duplications of the chromonemata without subsequent separation.

The synergids degenerate before fertilization, as in *A. arifolium*, and the first division of the zygote occurs at about the same endosperm stage as in that species. The nucellus, however, disappears at a much faster rate than in *A. arifolium*. It is practically gone by the time that the embryo is in the two-celled stage (Fig. 53).

The seed, although conforming to the *A. arifolium* pattern, differs in its general shape and in the structure of the seed coats. Instead of being turgid and circular in cross-section as in the other three species, it is flattened, with the endosperm and seed coat forming a U-shape around the funiculus (Fig. 55). The seed coat

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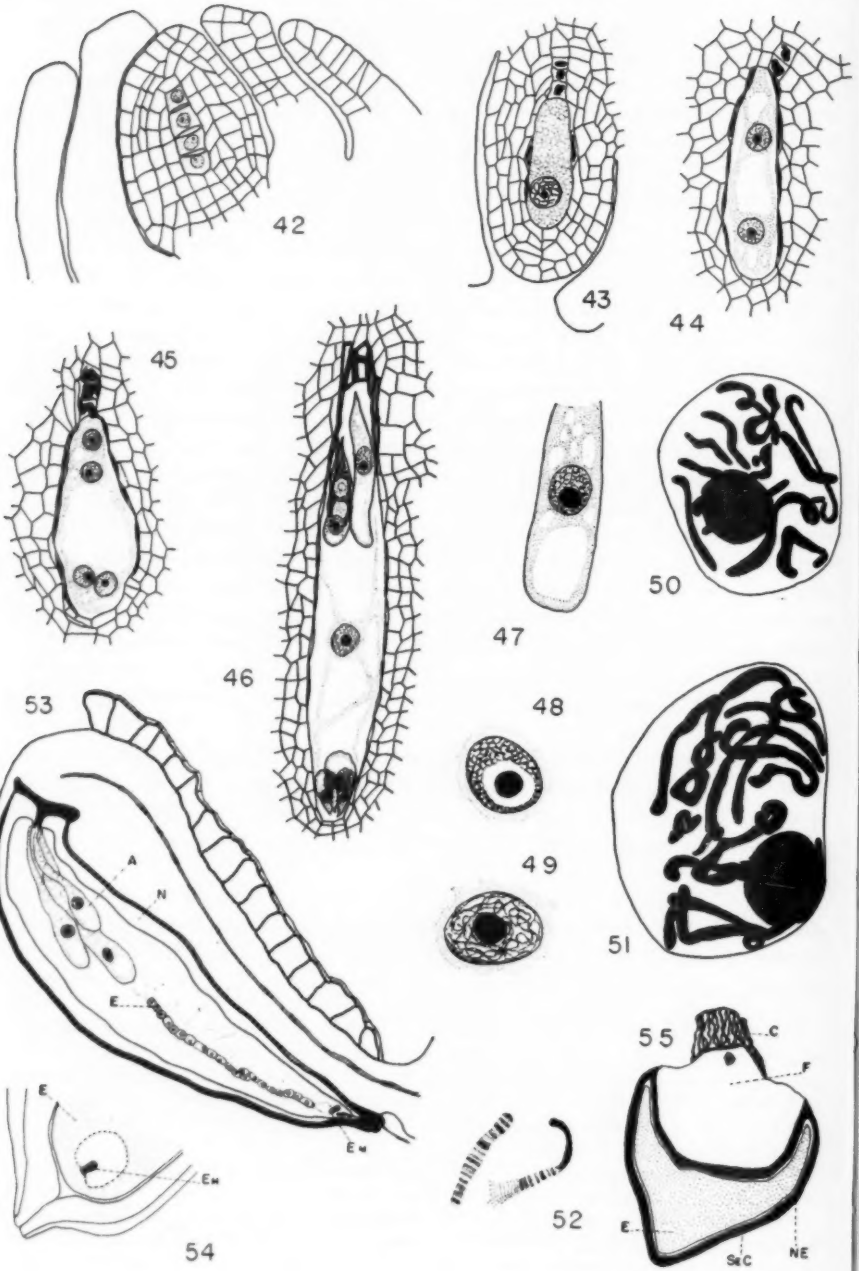
FIGS. 33–36. *Asarum virginicum*. FIG. 33. Longitudinal section of aberrant embryo sac with thirty-two nuclei. × 181. FIG. 34. Longitudinal section of aberrant embryo sac with thirty-two nuclei polarized. × 181. FIG. 35. Longitudinal section through wall of mature seed. × 300. *E*, endosperm; *NE*, nucellar epidermis; *ISC*, inner seed coat; *OCS*, outer seed coat. FIG. 36. Longitudinal section of mature seed. × 19. *C*, caruncle; *E*, endosperm; *NE*, nucellar epidermis; *Em*, embryo.

FIGS. 37, 38. *Asarum Lewisii*. FIG. 37. Longitudinal section of ovule prior to fertilization. × 69. FIG. 38. Longitudinal section of mature embryo sac. × 181.

FIGS. 39–41. *Asarum canadense*. FIG. 39. Longitudinal section of a young flower. × 19. *S*, stigma; *SC*, stylar canal; *Ca*, calyx; *P*, pollen sac; *O*, ovule. FIG. 40. Longitudinal section of ovule at time of fertilization. × 69. FIG. 41. Longitudinal section of inner integument and nucellus with megaspore mother cell. × 300.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is made up of five layers of cells. The first contains elongated cells with very thin walls; the second, large, cuboidal cells, thickened toward the inside; the third, elongated, thick-walled, fiber-like cells; the fourth (sometimes of 2 or 3 rows of cells) of sclerenchyma cells, very thick-walled; and the fifth, elongated, thin-walled, dark-staining cells. The nucellar epidermis is persistent as in the other species, and the antipodals have disappeared. The embryo is undifferentiated, but contains more cells than that of the other species. It is merely a spherical mass of dark-staining cells surrounded completely by endosperm (Fig. 54).

DISCUSSION

There is much confusion in present-day literature as to the phylogenetic position of the Aristolochiaceae. Various embryological features reported above for the genus *Asarum* indicate that the family is probably a relatively primitive one. The major primitive characteristics include (cf. Maheshwari, 1950) a stylar canal, anatropous ovules, two integuments, a massive nucellus, an archesporial cell hypodermal in original, division of the archesporial cell into a primary parietal cell and a primary sporogenous cell, a monosporic eight-nucleate embryo sac, cellular endosperm, delayed division of the zygote, and a massive endosperm.

The occurrence of polyteny, which has been suggested in the species studied, may also fit in with the indications of primitiveness suggested by Battaglia (1951). He states that "it is obvious that the antipodals are also potential endosperm initials, but are usually ineffective because the proendospermatic cell is favoured by size and nuclear number. It is natural, then, that in certain cases the antipodals may resume mitotic activity to produce a tissue of variable size (phenomenon of polyantipody). Such a tissue is the example of primary endosperm in angiosperms. This interpretation is supported by the observation of mitotic peculiarities during polyantipody (i.e., multinucleate cells, karyogamy, etc.), similar to those seen in the endosperm cells of *Gnetum* and *Welwitschia*. *Polyantipody, in the phylogeny of angiosperms, should, therefore, be an indication or primitiveness.* Such cases of polyantipody as have been reported are generally associated with the Normal type of embryo sac development. *Thus this last situation (normal type plus polyantipody) must be regarded as the most primitive*

FIGS. 42–55. *Asarum canadense*. FIG. 42. Longitudinal section of ovule with tetrad of megaspores. × 300. FIG. 43. Longitudinal section of nucellus with functional megaspore and remains of other three megaspores. × 300. FIG. 44. Longitudinal section of two-nucleate embryo sac. × 300. FIG. 45. Longitudinal section of four-nucleate embryo sac. × 300. FIG. 46. Longitudinal section of mature embryo sac after fusion of polar nuclei. × 300. FIG. 47. Longitudinal section of a portion of an antipodal cell at the eight-nucleate embryo sac stage. × 670. FIG. 48. Nucleus of antipodal cell at the seven-nucleate embryo sac stage. × 670. FIG. 49. Nucleus of antipodal cell immediately after entrance of the pollen tube into the embryo sac. × 670. FIG. 50. Nucleus of antipodal cell when embryo is in four-celled stage. × 670. FIG. 51. Nucleus of antipodal cell when endosperm is in the sixty-four-celled stage. × 670. FIG. 52. Portions of chromosomes from antipodal cell nucleus when endosperm is in the sixty-four-celled stage. × 670. FIG. 53. Longitudinal section of ovule after fertilization with large antipodal cells and early endosperm. × 19. *A*, antipodal cell; *N*, nucellus; *E*, endosperm; *Em*, embryo. FIG. 54. Longitudinal section of micropylar end of mature seed. × 69. *Em*, embryo. FIG. 55. Cross section of mature seed. × 19. *C*, caruncle; *F*, funiculus; *E*, endosperm; *SeC*, seed coat; *NE*, nucellar epidermis.

embryological behavior in angiosperms." Although polyteny in this case is not definitely polyantipody, it might be thought of as a degree of it, probably a step removed from it.

Most authors have associated the Aristolochiaceae with taxa which are considered by most systematists as primitive, although some (e.g. Small, 1933) have placed the family adjacent to the Cucurbitaceae. The family Aristolochiaceae, together with the Rafflesiaceae and Hydnoraceae, were treated by Solereder (1889) as belonging to the Order Aristolochiales. Bessey (1915) treated these three families not as a separate order, but as the most advanced taxon within his Myrtales, an order derived from the Rosales, with the Loasales and Cactales as the ultimate branches. Hallier (1905) accepted the Order Aristolochiales as set forth by Solereder, but added the Balanophoraceae to the order. He derived the order from the ranalian Lardizabalaceae, and considered the Rafflesiaceae as reduced parasitic Aristolochiaceae and the Hydnoraceae and Balanophoraceae derived from the Rafflesiaceae by two separate lines. Wettstein (1935) considered the three families of Solereder's Aristolochiales as derived from myrtaceous and annonaceous ancestors and included them within his order Polycarpicae, a taxon to which he assigned the Magnoliaceae as the primitive family. Hutchinson (1926) included the families Aristolochiaceae, Cytinaceae (Rafflesiaceae), Hydnoraceae, and Nepanthaceae in the order Aristolochiales. He considers that the order probably represents reduced Berberidales by way of Menispermaceae. He pointed out that this relationship, not obvious from a study of temperate Aristolochiaceae, becomes more evident from examination of such genera as *Saruma* with its conspicuous petals and separate pistils and its ranalian wood anatomy.

The view of Hutchinson is most widely accepted today. On the basis of embryology, a link with the Ranales is also indicated. The Beriberidaceae and *Asarum* have in common a small embryo, which is also characteristic of most of the Ranunculaceae and Magnoliaceae. The embryo in most of the species of these families is not as undifferentiated as it is in *Asarum*, however. Maheshwari (1950) states that this condition is found in the families Balanophoraceae, Rafflesiaceae, Gentianaceae, Pyrolaceae, Orobanchaceae, Burmanniaceae, and Orchidaceae, and appears to be associated in some degree with a parasitic or saprophytic mode of life. However, *Ranunculus ficaria* (Soueges, 1913) is not associated with a parasitic or saprophytic mode of life and has an undifferentiated embryo.

Members of the Ranales and *Asarum* have in common a copious fleshy endosperm, a nucellar cap at the micropylar end, an upper vacuole in the egg cell, a massive nucellus, two integuments, monosporic eight-nucleate embryo sac development, similar embryo development, cellular endosperm, a persistent nucellar epidermis, and large antipodal cells which often occupy about half the embryo sac. The last-mentioned similarity is very striking. Maheshwari (1950) reports that the antipodal cells of some members of the Ranunculaceae become greatly enlarged and assume a glandular appearance. Grafl (1941) has shown that in *Caltha palustris* they attain a high degree of polyploidy. Osterwalder (1898) showed the same thing for *Aconitum napellus*. Osterwalder did not suggest

polyteny, but from observation of his figures it is highly probable that such a condition exists.

SUMMARY

An embryological study of *Asarum arifolium* Michx., *A. virginicum* L., *A. Lewisii* Fern., and *A. canadense* L. var. *reflexum* (Bickn.) Robins., is reported.

All four species show a parallel development of the seed. They have in common a gynoecium of six carpels fused at the base, a stylar canal, anatropous ovules, two integuments, a massive nucellus, an hypostase, an archesporium of one cell and of hypodermal origin, a tetrad of megaspores, one of which becomes the functional megaspore, an eight-nucleate *Polygonum* type (normal) embryo sac, enlarged antipodal cells with nuclei suggesting polyteny, growth of the pollen tube through the stylar canal to the ovule, fusion of the polar nuclei some time before fertilization, a copious cellular endosperm, an undifferentiated embryo, a seed coat formed from the two integuments with similar modifications, a persistent nucellar epidermis, and a caruncle running the length of the funiculus.

Asarum virginicum differs from *A. arifolium* in having an ovary which is only very slightly inferior, no introrse stigmatic surface, a micropyle formed from the inner integument only, and synergids which are persistent through fertilization. One of the flowers of *A. virginicum* investigated showed ovules with an aberrant type of embryo sac development.

The development of the seed of *Asarum Lewisii* more nearly parallels that of *A. virginicum* than that of *A. arifolium*. The ovary is completely superior. The synergids are persistent and still present at fertilization and the micropyle is formed from the inner integument only.

Asarum canadense differs from the other three species studied in having a completely inferior ovary, fused styles and stigmas, a tetrad of megaspores in which the micropylar one is the functional megaspore, and very large antipodal cells with giant chromosomes showing distinct chromonemata and chromomeres, and a flattened seed.

Embryological investigations indicate that the embryo sac of *Asarum* is primitive and suggests derivation of *Asarum* and the Aristolochiaceae from some group such as the Ranales.

ACKNOWLEDGMENTS

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MORPHOLOGICAL STUDIES IN RHAMNACEAE

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I. INTRODUCTION

A series of important and interesting plant families, centering around and possibly allied to Celastraceae, possess as a more or less distinctive feature of their flowers, a disk. The morphologic nature of this structure, which in a single family or among a series of families is expressed in a variety of ways, is in need of

close study and evaluation. The orders Rhamnales, Olacales, Santalales, Rutales, Meliales, Sapindales, and the Umbelliflorae (in part) in some or all of their families possess this structure; and in the light of the general acceptance of the idea that these constitute a rather loose phyletic series, it is to be wondered whether the disk is an expression of a major morphologic trend, expressing itself through a series of patterns involving such features as perigyny and epigyny. To what extent and in what way the disk may be involved in the attainment of the epigynous condition, as well as the morphologic nature of the whole tissue-complex which envelopes the ovulary in the epigynous forms, are questions of prime import.

Genera from each of the principal tribes of the family have been studied: *Zizyphus* and *Berchemia* of the tribe Zizypheae; *Rhamnus* and *Ceanothus* from the tribe Rhamneae; *Colletia* from the tribe Colletieae; and *Gouania* from the tribe Gouanieae. In all, twelve species were examined in detail, either as microscopic serial sections or by the use of cleared whole flowers.

The working definitions proposed by Berkeley (1953 pp. 187–188) for his treatment of Celastraceae have been adopted in the present study.

Phylogenetically, Rhamnaceae occupy a position near Celastraceae. Hutchinson (1926) places the family as a direct descendant from Celastrales and indicates that perhaps Rhamnaceae gave rise to Myrsinales. Weberbauer (1895) and Suessenguth (1953) consider the closest relatives of Rhamnaceae to be in Vitaceae. Weberbauer suggests that perhaps the connecting link between these two families is the genus *Gouania* of Rhamnaceae. He also considers the tie between Rhamnaceae and Celastraceae as being very close.

A search of the literature has failed to reveal any systematic treatment of the floral morphology of the family, although Arora (1953) does mention some morphological characteristics of *Zizyphus* in a paper concerned primarily with the embryology of *Zizyphus rotundifolia* Lam.

II. MATERIALS AND METHODS

The family Rhamnaceae is divided by Suessenguth (1953) into five tribes: Rhamneae, Zizypheae, Ventilagineae, Colletieae, and Gouanieae. This tribal classification is based primarily on the type of fruit produced. Fresh material of the following genera was available in the Chapel Hill area: *Zizyphus*, *Berchemia*, *Rhamnus*, and *Ceanothus*. This was supplemented with herbarium material of *Colletia* and *Gouania*, these six genera representing all the tribes. Buds, flowers, and fruits were collected at frequent intervals throughout the year, preserved in FPA fixative (commercial formalin 5 parts, propionic acid 5 parts, and 50% ethyl alcohol 90 parts), stored in the fixative until needed for study, then dehydrated by the tertiary butyl alcohol method and embedded in paraffin.

Herbarium material (flowers) were aspirated in a warm 1% solution of sodium hydroxide and allowed to soak for 5 hours, washed 24 hours in running water and paraffin-embedded by the tertiary butyl alcohol method.

All drawings of sectioned material were made with the aid of a camera lucida. Whole flowers were drawn free-hand with the use of a dissecting microscope.

Some of the flowers which had been stored in a 1:1 mixture of paraffin oil

and tertiary butyl alcohol were later cleared and the vascular anatomy of the whole flower studied. In some instances, the fresh shoot was immersed in Gourley's stain, immediately after cutting, for 18 hours, washed in running water for 1 hour and placed in 50% alcohol for 12 hours. After a chlorine bleaching the material was dehydrated and cleared in xylol where it was left indefinitely. The vascular tissue stained very well. Flowers of greater bulk require longer treatment.

III. Gross Morphology of the Flower

The calyx consists of 4 or 5 more or less pointed triangular sepals, valvate in the bud. While quite variable in form, the sepals are, nevertheless, characteristic of the family. In most members of the family the inside of the sepals is covered by a fleshy and glossy layer which ends as a fleshy tubercle toward the extremity of the sepals. This type of thickening of the sepals appears to be limited largely to the family Rhamnaceae. There is no histologic difference in the tissue of the disk and this fleshy layer of the sepals. The petals are often small and inconspicuous. They are usually clawed and hooded and enclose the stamens. Their point of insertion is on the tube, alternate with the sepals. In those genera with a prominent disk, the petals are inserted at the border between the disk and the calyx, but never on the disk. Where the disk is limited to the bottom of the tube, as in *Colletia*, the petals, if present, are inserted on the calyx. The stamens, with versatile, usually introrse anthers, are opposite the petals. The single pistil is composed of 2–4 carpels. The species reported on in this study were bi- or tricarpellate. The ovulary may be superior or partly to wholly inferior, with 2–4 locules each with 1 (rarely 2) anatropous ovules. Placentation is basal. The fruit may be drupaceous, capsular, or rarely samaroid. The embryo is large and straight in sparse fleshy endosperm. The cymose nature of the inflorescence may be quite evident in some genera, while in others it has been obscured by reduction.

No structure presents a greater range of expression or is more important for separation of the genera than the disk. Since the disk occupies a central position in this study, a comparative treatment of its gross features and variety of expressions is deemed important at this point. In all genera it is more or less extensively adnate to the tube. Sometimes, however, it covers only the bottom of the floral tube. The genera can be grouped, according to the expression of the disk, into four categories:

1. In *Rhamnus*, *Sageretia*, and *Scutia* the disk extends up into the floral tube, its form depending upon the shape of the tube. Usually it is urecolate, surrounding the ovulary more or less closely, but never adhering to it. In *Rhamnus* the disk is fused with the floral tube for the entire length of the disk, while in *Sageretia* the disk may be free from the floral tube for the entire length of the disk.

2. In *Paliurus*, *Hovenia*, and *Colubrina*, the disk fills the cavity of the floral tube and encloses the ovulary. It adheres in part to the ovulary and appears as a large, flat, pentagonal ring. This partial adherence of the disk to the ovulary seems to determine the persistent adherence of the tube to the fruit.

TABLE I

Various expressions of the disk according to the present tribal arrangement of the family

	Rhamneae	Zizypheae	Gouanieae	Colletieae
Disk conforms to shape of floral tube, free from ovulary	X			
Disk fills cavity of the floral tube, partly adnate to the ovulary	X	X		
Disk fills cavity of the floral tube, almost completely adnate to ovulary	X	X	X	
Disk apparently lacking	X			X

3. In *Zizyphus*, *Gouania*, and *Ceanothus* the disk is almost or completely adherent to the ovulary. In these genera the disk not only serves as a means of union between the calyx and ovulary, but covers the ovulary in many instances with a thick layer, thus forming a very distinct epigynous disk.

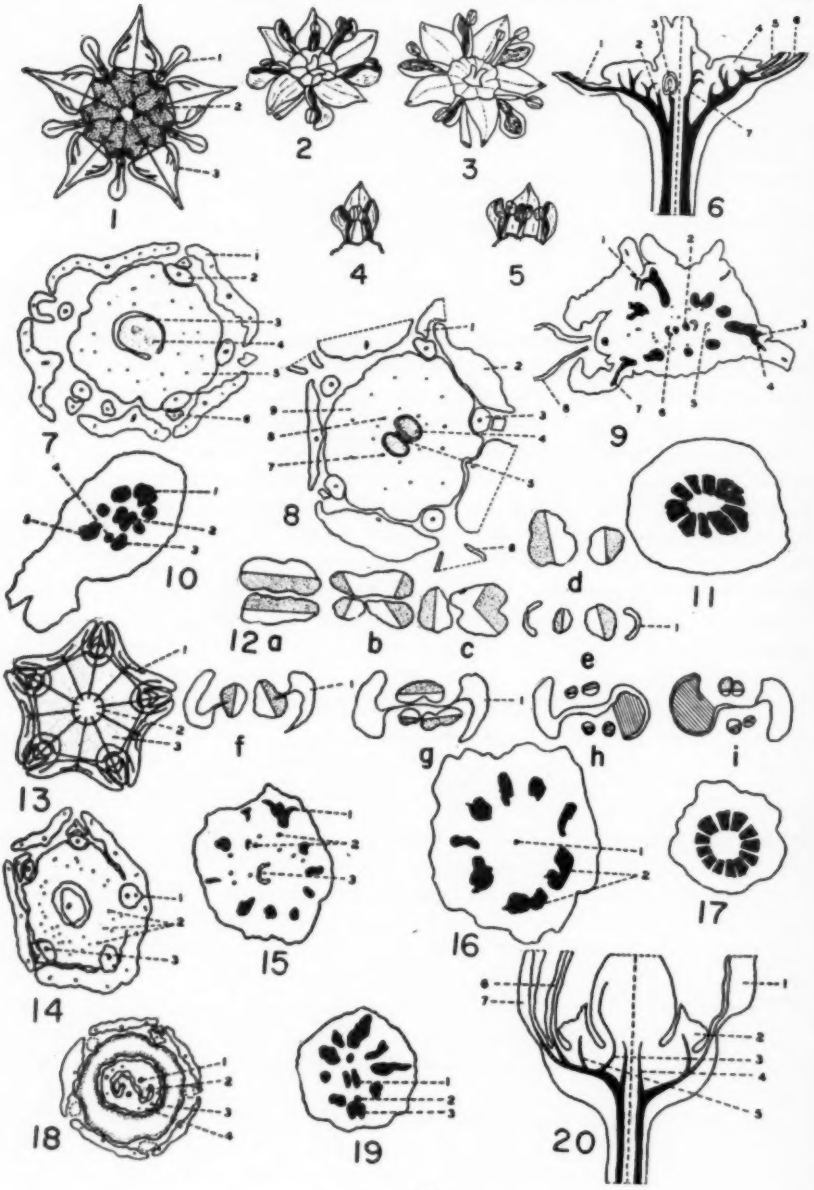
4. The disk appears to be completely lacking in other genera: *Phylica*, *Retanilla*, *Cryptandra*, and *Pomaderris*. At least in these genera one does not readily see the thick fleshy layer which covers a limited part of the calyx or ovulary. It seems, however, that it is not a case of absence of the disk, but that the disk coats the calyx from its base up to the extremities of the sepals. In these genera the disk is actually a thick, fleshy layer, clearly distinct from the tissue of the calyx or else the disk has been reduced to such a thin layer that it is recognized only as the peculiar-looking, waxy, internal surface of the calyx.

IV. Floral Morphology and Anatomy

A. *Zisyphus* Mill.

The flowers of *Zizyphus* are small, yellow, white or greenish, and typically 5-merous. A number of variations, however, have been found in the flowers from a tree growing in the Coker Arboretum at Chapel Hill. These variations are: 6 stamens with the extra stamen opposite a sepal; 2 extra stamens with fused filaments and 2 anthers opposite a sepal; an extra stamen opposite a sepal with petal-like appendages; and a variation from 4–8 parts (Figs. 2–5). The flowers are perfect. There are usually 5 sepals with a prominent keel down the inner mid-line with a distinct tubercle at the distal end of the keel. The limb of the short-clawed petal is oval or sub-orbicular and hood-like. The ovulary is 2-loculed, each locule with one basal ovule, and is immersed in the prominent disk. The fruit is an ovoid or ellipsoid fleshy drupe. The flowers are borne in short axillary cymes.

This genus is the largest of the Tribe Zizypheae (100 species) and according to Suessenguth (1953) is mainly Indomalaysian, with a few species in tropical and southern Africa, some in eastern Asia and Australia, as well as South America. The writer has examined specimens in the National Herbarium from 7 states of the United States (southern, southwestern, and west coast); 27 countries of Central and South America; widely scattered areas of Africa; many regions of Asia including India, China, Manchuria, and Japan; the east Indies and the Phil-



FIGS. 1–20

ippines. This indicates a somewhat wider distribution than that stated by Suessenguth.

Zizyphus jujuba Mill.—The vascular tissue in the pedicel is greatly dissected, the vascular elements being divided by rows of parenchyma cells (Fig. 11). In the lower part of the receptacle, traces to the perianth begin to move out. Usually there are 7 distinct, well-defined traces at this level which will supply sepals, petals, and stamens. Three of these traces are larger than the other 4 and contain traces of petal-stamens and sepals as well as carpel dorsals (Figs. 1, 10). As these traces move out from the stele they leave stelar tissue behind which will eventually become carpel ventrals. The perianth traces move outward and

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FIGS. 1–12. *Zizyphus jujuba* Mill. FIG. 1. Diagram of perianth supply projected on to a horizontal plane. 1, Petal; 2, Disk; 3, Sepal. FIGS. 2–5. Free hand sketches of abnormal flowers. FIG. 2. Stamen and petal opposite a sepal. FIG. 3. Extra stamen almost opposite a sepal. FIG. 4. Two stamens with fused filaments opposite sepal. FIG. 5. Petaloid stamen opposite sepal. FIG. 6. Diagram of longitudinal section of flower, the left half a radial plane through the sepal, the right half the same through the petal. 1, Petal; 2, Carpel dorsal; 3, Ovule; 4, Disk; 5, Stamen; 6, Petal; 7, Carpel dorsal giving off branches into ovulary wall. FIGS. 7–11. Cross sections through a single flower. FIG. 7. At level where disk becomes free from style and perianth members. 1, Sepal; 2, Stamen; 3, Style; 4, Transmitting tissue of style; 5, Disk; 6, Claw of petal. × 11. FIG. 8. Middle of ovulary. 1, Claw of petal; 2, Sepal; 3, Stamen; 4, Ovule; 5, Carpel ventral; 6, Part of petal; 7, Carpel dorsal; 8, Carpel lateral; 9, Disk. × 16. FIG. 9. At base of locules. This section slightly oblique, being lower at left than right. 1, Sepal lateral departing from petal-stamen trace; 2, Carpel ventral; 3, Petal-stamen trace; 4, Sepal lateral; 5, Carpel dorsal; 6, Locule; 7, Sepal lateral; 8, Petal. × 16. FIG. 10. Through receptacle. 1, Fused mid sepal-petal-stamen traces; 2, Residual stelar tissue; 3, Mid sepal trace; 4, Carpel dorsal; 5, Petal-stamen trace. × 16. FIG. 11. Through stele before departure of the floral traces. × 29. FIG. 12, a-i. Series of drawings showing behavior of carpel ventrals. Stippled areas indicate xylem, hatched areas ovules. 1, Locule. All figures × 41. a, Stelar tissue which will furnish carpel ventrals; b, Xylem has moved to end of bundles; c, Bundles split through phloem; d, Separate bundles with xylem moved 90 degrees from normal position in the stele; e, Locules of ovulary beginning to form; f, Locules cutting the vascular tissue; g, Vascular tissue breaking up and reuniting; h, Carpel ventrals with xylem rotated 180 degrees from its position in the stele; ovule forming; i, Section slightly higher than "h" showing more advanced ovule.

FIGS. 13–20. *Berchemia scandens* (Hill) K. Koch. FIG. 13. Diagram of perianth supply projected on to a single horizontal plane. 1, Lateral branches from mid sepal trace; 2, Inner limits of disk; 3, Disk. FIGS. 14–17. Cross sections through a single staminate flower. FIG. 14. At level where petals, stamens, and sepals become free, slightly oblique. 1, Petal-stamen tissue still fused; 2, Many small vascular traces in the tissue of the disk; 3, Petal becoming free from the stamen. × 11. FIG. 15. At base of rudimentary pistil, slightly oblique. 1, Sepal lateral departing from petal-stamen trace; 2, Traces to wall of rudimentary ovulary; 3, Rudimentary pistil (style) becoming free. × 11. FIG. 16. Through receptacle. 1, Small ventral carpel trace; 2, Fused petal-stamen and mid sepal traces. × 27. FIG. 17. Through stele before departure of floral traces. × 26. FIG. 18–19. Cross sections through a single pistillate flower, both sections × 11. FIG. 18. At base of the locules. 1, Carpel ventral; 2, Ovule just starting to form; 3, Carpel dorsal; 4, Disk showing heavy staining cells on its inner and outer edges. × 11. FIG. 19. Through receptacle, slightly oblique, lower at the left. 1, Carpel ventral; 2, Carpel dorsal; 3, A petal-stamen and sepal midrib trace fused. × 11. FIG. 20. Longitudinal section of the pistillate flower, left half in radial plane through a petal midrib, right half in plane of sepal midrib. 1, Sepal; 2, Disk; 3, Carpel dorsal; 3, Carpel ventral; 4, Carpel ventral; 5, Vascular stub into disk; 6, Filament; 7, Petal.

upward rather quickly since the axis of this flower has been greatly shortened. Each of the 3 large traces (Fig. 10) gives off traces to petal-stamens and to sepals. The carpel dorsals come from 2 sepal traces at opposite ends of the receptacle. At the base of the locules of the ovulary the traces split into 10 traces and from these 10 other traces split off (Fig. 9). At this level, i.e. the base of the locules, the petal-stamen traces begin to split, sending traces to the lateral margin of the sepals, the remaining vascular tissue continuing on into the stamen and petal. Smaller branches come off from all the perianth traces and move up into the disk to end blindly (Figs. 6, 7). The stelar tissue left when the perianth traces moved out continues upward and gives off the carpel ventrals.

The behavior of the stelar tissue which will become the carpel ventrals is peculiar and not a little puzzling in comparison with other genera of the family. Below the base of the locule the xylem and phloem are normally oriented as in the stele, i.e. the phloem is toward the outside of the receptacle while the xylem is on the inside (Fig. 12, a). Just before reaching the base of the locules the xylem moves through 90 degrees and the stelar traces begin to break up (Fig. 12, b–e.). As the carpels become distinct they cut through both these bundles (Fig. 12, f). Concurrently with this splitting there is another 90-degree movement of the xylem and phloem so that at the level at which the ovules form there has been a complete inversion of the conducting tissue (Fig. 12, g). Then each of the large bundles breaks up into 2, which are the carpel ventrals (Fig. 12, h). Near the level of the middle of the ovulary these 2 fuse again into 1 (Figs. 8; 12, i). In the meantime the carpel dorsals, which are fused with the sepal traces in the receptacle, give off small traces which move up into the ovulary wall, some to end blindly, others to become carpel laterals (Fig. 6).

Between the middle and the top of the ovulary, all the perianth members become free (Figs. 7, 8). This feature seems to depend on the stage of development, the older (in ontogeny) the flower, the nearer the middle of the ovulary the perianth parts become free. At this level there is no visible difference between the tissue of the ovulary wall and that of the disk. The approximate limits of the ovulary wall can be made out only by the location of what this writer interprets to be the carpel dorsals and carpel laterals. By the time the style separates from the disk, however, all the perianth members become free (Fig. 7). In the style, just above the top of the locule, the carpel traces divide into four distinct bodies of vascular tissue. They gradually fade out in the tissue of the style. At the approximate top of the locule there is a splitting and slight downward turning of the dorsal carpel trace as seen in the reconstructed longitudinal view of the flower (Fig. 6). This trace, however, is not evident in cross section. Well defined stigmatoid tissue (Esau, 1953) is present throughout the remainder of the style up to the stigma. Crystals are quite common in the cells of the disk. As the traces to the petal-stamens, as well as those to the sepals, move out and up through the disk, they give off, near the edge of the disk, small lateral branches which move laterally and upward (Figs. 1, 6).

B. *Berchemia* Neck.

Some manuals describe the flowers of *Berchemia* as being perfect (Gleason, 1952), while others describe them as being perfect or polygamous (Small, 1953; Rehder, 1940). In all the material collected by this writer in the Coastal plain of North Carolina, only staminate flowers were found. Very few pistillate or perfect flowers were found in herbarium material, although many specimens were seen with fruit.

The flowers are small, 5-merous, greenish white, in terminal or axillary panicles. The calyx has a very short or roundish tube, the flat to slightly triangular sepals a little longer than the petals. The petals are oblong, acute, sessile, narrower than the sepals, their margins involute around the filaments of the stamens. The disk is very thick and flat, filling the tube, surrounding, but not fused with, the ovulary. The stamens are often shorter than and opposite the petals, being enclosed by the latter. The pistil is made up of a 2-celled ovulary, short undivided style, and stigma. The fruit is an ellipsoid or ovoid, somewhat flattened, drupe, with a thin, leathery, bluish-black pericarp, 6–8 mm. long, with a 2-celled stone.

There are 22 species of *Berchemia*, rather widely scattered. In the course of this study the writer has examined herbarium specimens from 12 southeastern and southwestern states ranging from Virginia to Florida and westward to Oklahoma, Missouri, and Texas. Specimens were also studied from Guatemala, various provinces of China, French Indo-China, India, Formosa, Okinawa, Japan, New Zealand, Africa and Luzon, P. I.

B. scandens (Hill) K. Koch.—In the staminate flower the stele of the pedicel is dissected (Fig. 17). Traces to the petals, stamens, and sepals depart from the stele in the receptacle, all at about the same level. At this point the petal and stamen traces are not distinct from each other. A cross section of the receptacle at this level shows 8 distinct traces with 2 of these being composed of united sepal and petal-stamen traces (Fig. 16), while the carpel dorsals are fused with the perianth traces in varying degree. A very small ventral carpel trace moves inward from the stele, turns upward, and fades out before the base of the rudimentary ovulary is reached (Fig. 16). As the perianth traces move outward through the receptacle, smaller traces depart from them and move upward. These are the dorsal and lateral traces to the rudimentary carpels. It is impossible to distinguish carpel dorsals from carpel laterals in the staminate flower since the ovulary does not develop (Fig. 15). Traces also come off the petal-stamen traces, move out into the tissue of the receptacle, move up and end blindly in the tissue of the disk. These traces are largely protoxylem (Fig. 14). At the level where the rudimentary style (ovulary) becomes distinct, sepal laterals split off radially and move through the tissue to the lateral margin of adjacent sepals, bend sharply and move up into the sepal (Fig. 15). Just above this point the stamen trace separates tangentially from the petal trace. This tissue of the stamen and petal remains undifferentiated until about the point at which the disk becomes distinct from the perianth (Fig. 14). The traces to the sepal midribs have moved

into the sepals and have turned sharply upward. Two branches come off the mid-sepal trace, each moving laterally from the midrib (Fig. 13).

In the perfect flower the vascular supply to the perianth differs little from that of the staminate flower. In the receptacle, however, there is a difference due to the vascular supply to the carpels. The carpel dorsals are fused with the petal-stamen traces and move out some distance with these traces before they split tangentially from the inner side and move up into the wall of the ovulary (Figs. 19, 20). The rest of the stele above the level at which the perianth supply departs becomes the 2 well-developed ventrals (Figs. 18, 19). These move into the base of the locules, give off short placentals, and then fade out very shortly in the septum (Figs. 18, 20). Just below the level at which the petal-stamen trace splits, a well-defined trace departs, moves into the tissue of the disk and ends blindly (Fig. 20). The disk becomes free from the perianth as well as from the ovulary at the approximate level of the base of the locules (Fig. 18). The ovulary is 2-celled with a single basal ovule in each cell. The 2 carpels are incompletely fused in the lower part of the ovulary. The cells of the inner and outer limits of the disk are very conspicuous due to abundant cytoplasm (Fig. 18).

C. *Rhamnus* L.

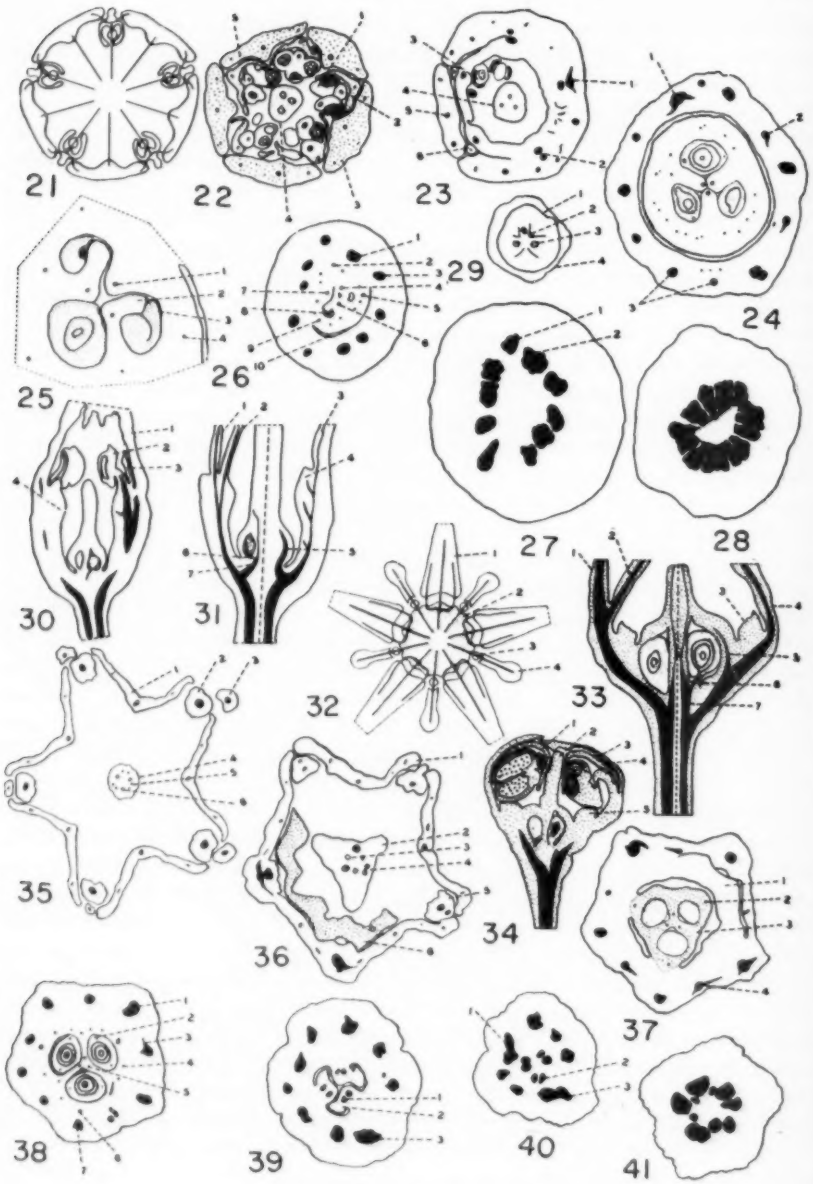
The flowers of Rhamnus are small, greenish, yellowish, or whitish, perfect or with either stamens or pistils, 4- or 5-merous, and borne in axillary clusters, umbels, or racemes. Sepals are united at the base, and together with the fused bases of the stamens and petals form the tube. The petals are very small, short-clawed, obcordate, and are inserted on the disk which lines the tube (Figs. 23, 30). In at least 1 species (*R. alnifolia* L'Her) petals are wanting. The stamens with acute anthers are opposite the petals and included by them. In one specimen of *R. caroliniana* Walt. 6 sepals, petals, and stamens were found. The 2–4-loculed ovulary is free from the disk. The style is usually undivided. The fruit is a drupe (berry-like and red, turning to black) with 2 to 4 seed-like nutlets of cartilaginous texture embedded in the succulent flesh.

The genus contains about 155 species, widely distributed, expecially in temperate and subtropical climates. A few species range into the tropics. The genus is found in North and South America, Australia, Polynesia, China, and all parts of Europe. In this study, material was studied from 5 provinces of Canada, 41 states of the United States, 11 countries of Central America, 7 countries of South America, 12 European countries, 4 countries of Asia, 10 provinces of Africa, the Philippines, British New Guinea (Papua), Java, East Coast of Sumatra, Toba, and Fiji.

Two species of *Rhamnus* are included in this study, *viz. R. caroliniana* Walt. and *R. frangula* L. Since the vascular plan of *R. frangula* L. was found to be essentially the same as *R. caroliniana* Walt., only the latter is reported on in detail.

R. caroliniana Walt.—In cross section the pedicel displays a dissected stele (Fig. 28). At the base of the receptacle the stele begins to lose its highly-dissected nature and forms into distinct traces. The traces destined for the petals-stamens, sepals, and carpels are variously fused at this level, and all of them

leave the stele at the same level (Fig. 27). The tissue of the receptacle outside the stele is highly secretory. The carpel traces, fused with the petal-stamen and sepal traces, bend outward with them, and then upwards into the ovulary (Fig. 31). The vascular supply in this species is a little peculiar when compared with other members of Rhamnaceae. Near the level of the middle of the receptacle, 10 traces depart from the stele and move outward. No stelar tissue is left. The ventral carpel traces leave first, i.e. lower down. One carpel ventral consists of 3 traces fused together. Two of these are fused with mid-sepal traces lower down and 1 with a petal trace. The other 2 carpels receive their ventral supplies from the petal traces (two from each petal). After the ventrals are given off, the dorsals arise. Here again the pattern is not as uniform as would be expected —2 dorsals are fused with the mid-sepal traces for a greater distance than the ventrals. At the level of the base of the locules, at which point carpel identity first appears, the 5 petal-stamen and 5 sepal traces become distinct. The ventral carpel traces are beginning their upward course, while the laterals as well as the dorsals are barely distinguishable from each other. It is at this level that the ovulary begins to separate from the tube (Figs. 26, 31). In a section through the flower about half-way up the ovulary from the base of the locules the petal-stamen and sepal traces are continuing their course up the tube (Fig. 23). There is much branching from the perianth bundles, especially the mid-sepal traces, into the disk. Throughout the length of the tube the disk is distinguishable from other tissue only by reason of its richly cytoplasmic cells. The ovules have well-defined vascular bundles. At this level, i.e. half-way up the ovulary, the ventral carpel traces have become more definite, but there is still little visible difference between the carpel dorsals and laterals (Fig. 25). Slightly above this level there is complete concrescence of the carpels. In the tube between this level and the top of the locule short traces are given off from the petal-stamen traces, some of which turn inward into the disk while others go around the tube (Fig. 24). Small lateral branches also come off the sepal traces. At this level the petal-stamen traces are larger than the sepal traces. Above the top of the locules the ventral carpel traces split, move toward the edges of the style and fade out in the tissue of the style (Figs. 29 and 31). There are 3 distinct stylar canals which are present the entire length of the style (Fig. 29). As the petal-stamen traces continue up through the tube, they give off short branches which move into the tissue of the disk and end blindly. Somewhat above the upper limits of the ovulary, but before any division appears between the tissue of the disk and the sepals and petals, a tangential split in the petal-stamen trace becomes evident (Figs. 23 and 31). After the petal traces separate from the stamen trace, two lateral traces are given off which move out to the margin of the tissue which will become the sepal (Figs. 21 and 23). At about this level the sepal trace breaks up, and branches move laterally into the future sepal tissue. About half way up the style the disk begins to separate from the petals and sepals (Fig. 23). The sepals become free from the disk slightly before the petals, thus leaving the petals and stamens as part of the disk with these tissues being indistinguishable from disk tissue. At this level the stamen trace is much larger than that of the petal. In the petals there are 3 distinct traces, a mid-sepal trace and 2 laterals.



FIGS. 21–41

The amount of secretory tissue has decreased considerably in the sepals. In the style the carpel traces have faded out but the three stylar canals are still quite distinct. Some distance above the point at which the petal and stamen traces split, a tangential split separates petal tissue from stamen tissue, leaving the stamen as a very definite part of the tissue of the disk. The short filament becomes distinct from the disk and supports the 2-lobed, 4-locular anther. The petals, with one bundle, enclose the anthers (Fig. 22). Sepals, in comparison with the petals, are massive.

D. *CEANOTHUS* L.

Flowers of this genus are small, perfect, regular, white (many of the western species are brightly colored), borne in terminal and axillary slender-peduncled

FIGS. 21–31. *Rhamnus caroliniana* Walt. FIG. 21. Diagram of perianth supply projected on a horizontal plane. FIGS. 22–28. Cross sections through a single flower. FIG. 22. At level where sepals, petals, stamens, and style have become free, before anthesis. Slightly oblique, left side being lower than the right. 1, Anther; 2, Petal clasping anther; 3, Sepal; 4, Style; 5, Connective of anther. × 16. FIG. 23. Above the ovulary, somewhat oblique, the left side higher than the right. 1, Petal trace with sepal laterals moving out; 2, Petal trace splitting from stamen trace; 3, Anther; 4, Style; 5, Sepal midrib; 6, Claw of petal. × 16. FIG. 24. About half-way up the ovulary. 1, Petal trace with lateral branches; 2, Sepal trace with lateral branches; 3, Traces to an extra petal and sepal. × 27. FIG. 25. Just above the base of locule showing only part of ovulary. Slightly oblique, the left side being higher. 1, Carpel ventral; 2, Lateral margin of carpel; 3, Ovule; 4, Carpel dorsal. × 41. FIG. 26. At base of locule, somewhat oblique, the right side being higher. 1, Petal trace; 2, Carpel dorsal; 3, Sepal trace; 4, Carpel ventral; 5, Carpel dorsal; 6, 7, Carpel ventrals; 8, Locule appearing; 9, Carpel dorsal; 10, Tube becoming free from ovulary. × 16. FIG. 27. At base of receptacle. 1, Petal trace; 2, Sepal trace. × 41. FIG. 28. Through pedicel before departure of floral traces. × 41. FIG. 29. Cross section of style. Only style and inner limits of disk shown. 1, Style; 2, Carpel ventrals splitting; 3, Stylar canal; 4, Inner limits of the disk. × 27. FIG. 30. Longitudinal section of flower before anthesis. 1, Sepal; 2, Anther; 3, Edge of a petal; 4, Disk. × 9. FIG. 31. Diagram of longitudinal section of the flower, the left half radial plane through petal-stamen; the right half the same through sepal. 1, Petal; 2, Stamen; 3, Sepal; 4, Disk; 5, Carpel dorsal; 6, Ovular trace; 7, Carpel ventral.

FIGS. 32–41. *Ceanothus americanus* L. FIG. 32. Diagram of perianth and disk supply projected on to a horizontal plane. 1, Sepal; 2, Disk; 3, Filament of stamen; 4, Petal. FIG. 33. Diagram of longitudinal section of flower, the left half in radial plane through petal midrib, the right half through a sepal midrib. 1, Petal, 2, Stamen; 3, Disk; 4, Sepal; 4, Carpel dorsal; 6, Ovular trace; 7, Carpel ventral. FIG. 34. Longitudinal section of a bud. 1, Anther; 2, Style; 3, Sepal; 4, Petal; 5, Disk. × 16. FIG. 35–41. Cross sections through a single flower. FIG. 35. At level where all whorls are free. 1, Sepal; 2, Stamen; 3, Petal; 4, Style; 5, Carpel ventrals which have separated into pairs; 6, Stylar canals. × 19. FIG. 36. Some distance above top of ovulary, slightly oblique, the left side being lower than right. 1, Lateral edge of sepal; 2, Style; 3, Stylar canal; 4, Carpel ventrals separating; 5, Petal-stamen; 6, Disk. × 19. FIG. 37. About three-fourths of the way up the ovulary. 1, Disk; 2, Carpel dorsal; 3, Wall of ovulary; 4, Mid sepal trace with a lateral branching off. × 16. FIG. 38. About half way up the ovulary. 1, Petal trace; 2, Ovular bundle; 3, Lateral departing from mid sepal trace; 4, Lateral trace to ovulary wall; 5, Carpel ventral; 6, Carpel dorsal; 7, Petal trace. × 16. FIG. 39. Through ovulary at base of locules. 1, Carpel ventral; 2, Ovules just forming; 3, Fused petal-stamen and sepal traces. × 26. FIG. 40. Through lower part of receptacle. 1, Fused perianth traces; 2, Carpel ventrals; 3, Fused perianth traces. × 26. FIG. 41. Through pedicel before departure of floral traces. × 41.

panicles. The lower peduncles are progressively longer, up to decimeters long, and are naked or have a few subterminal bracts. There are 5 sepals, inflexed, keel-less, and united at the base with the urn-shaped receptacle to form the tube. The 5 petals are distinct, hooded, long-clawed, incurved in the bud with the infolded sides of the broad blade clasping the anther (Fig. 34), but at anthesis widely spreading or deflexed between the sepals. There are 5 stamens opposite the petals which at anthesis are free and exserted. The filaments are elongated, the anthers emarginate. McMinn (1939) and Gleason (1952) describe the ovulary as being immersed in the annular disk. Bailey (1949) considers the ovulary to be immersed in the disk and adnate to it. In this study the ovulary has never been found to be completely immersed in the disk except in those specimens examined prior to anthesis (Fig. 34). The ovulary is 3-loculed, 3-lobed, with a short 3-lobed style. The fruit is a depressed-obovoid, 3-lobed capsule, the receptacle persisting as a cup.

All species of this genus are confined to the continent of North America with the center of distribution considered to be California (Van Rensselaer 1942). In his revision of the genus McMinn (Van Rensselaer 1942) includes 55 species, 25 varieties, 11 named natural hybrids, and several minor variations.

While the vascular tissue of only one species (*C. americanus* L.) was studied in detail, cleared flower specimens of the following species were studied: *C. divaricatus* Nutt., *C. Lemmonni* Parry, *C. ovatus* Desf., *C. cordulatus* Kell., *C. foliosus* Parry. The vascular pattern of these species was in general similar to that of *C. americanus* L.

C. americanus L.—The vascular tissue is organized in the pedicel in more or less discrete bundles. Seven or 8 rather large bundles as well as 2 or 3 smaller ones are usually distinguishable (Fig. 41). In the lower part of the receptacle the traces to the perianth separate from the ventral carpellary traces and the former move outward rather sharply, while the latter turn inward very slightly and move on upward to the carpels (Fig. 33). At this level there is still some fusion of perianth traces, but the ventral carpel traces are distinct from each other, there being two to each carpel (Figs. 40, 41). At the level of the base of the locules of the ovulary, the perianth traces become distinct with the exception of one which represents the union of elements destined for a petal-stamen and the mid vein of a sepal, there being 9 distinct traces at this level (Fig. 39). Also at this level there is incomplete concresence of the carpels. There is still no distinction to be made between the tissue of the ovulary and that of the disk. At about the middle of the ovulary all the perianth traces become distinct and move further from the center of the flower (Fig. 38). At this level the perianth traces continue to move upward with little evidence of lateral movement. Ten well-defined perianth traces show up here. The traces destined for the petals and stamens are fused together here and alternate with those which will supply the midrib of the sepals. (Fig. 32). Sepal laterals branch off at this level from the mid sepal trace (Fig. 38). Just below the level of the middle of the ovulary 1 dorsal carpel trace splits off from a petal-stamen trace and the other 2 carpel dorsals split from the sepal traces. At this level there is complete concresence

of the carpels. The union of carpels no doubt occurs during ontogeny, as various stages of concrescence have been noted. The ventral carpel traces unite at this level so that now there is only one trace to each carpel. The wall of the ovulary can be distinguished from the tissue of the disk by the smaller, more compact cells of the ovulary wall as contrasted to the highly glandular tissue of the disk. It is also possible to distinguish the perianth tissue from the tissue of the disk at this level. There are no crystalline inclusions in the cells of this species as are common in certain other genera of this family.

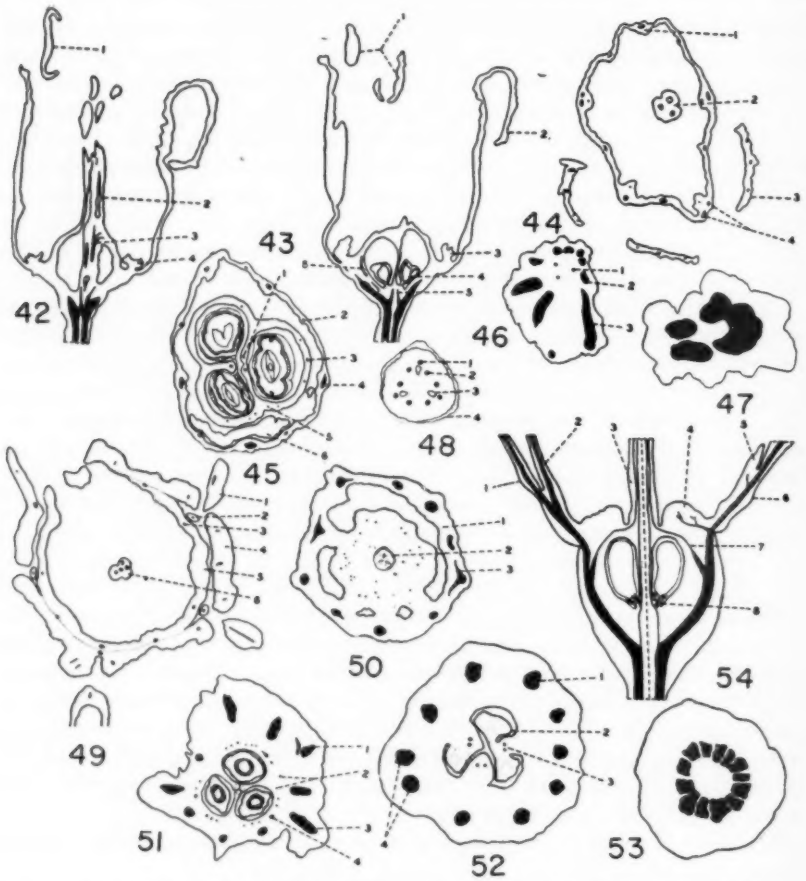
The disk begins to separate from the ovulary about three-fourths of the distance up the ovulary (Fig. 37) and completely separates from the wall of the ovulary before it separates from the petals, stamens, and sepals (Figs. 33 and 36). At about this level the sepals become distinct from each other but the tissue of the stamens and petals has not begun to separate. The petals and stamens become distinct from each other slightly above the top of the ovulary at about the level at which the single style becomes well defined. There are 3 small, but well-defined, stylar canals. These extend all the way up the style to the stigmas (Figs. 35, 36). At the base of the style the 3 ventral carpel traces are present as small bundles, but as these progress up the style they each split so that near the top of the style they become 6 small bundles in pairs (Fig. 36).

E. *Colletia* Juss.

The flowers are perfect, usually 4–6-merous, greenish-white, yellowish-white to white, borne on nodding 1-flowered pedicels, either solitary or fascicled. Small petals are borne at the top of the urn-shaped tube or are wanting in some species. Quite frequently the sepals are reflexed. The disk is inconspicuous, partially filling the base of the tube, and its margin in many cases is rolled in. The ovulary is immersed in the disk to varying degrees, contains 3 locules, each with a single, basal, anatropous ovule. The fruit is a dry, leathery, 3-lobed capsule which separates into 3 nutlets at maturity.

The genus is composed of 17 species of spiny shrubs confined to extratropical South America.

C. crenata Clos.—The vascular tissue of the stele in the pedicel occurs as 3 large bundles (Fig. 47). Each of the small bundles represents 2 fused bundles, while the larger one is made up of 6 bundles. The receptacle is severely shortened and as a consequence the traces move out from the stele rather abruptly forming 10 distinct traces (Fig. 46). Five of these traces are sepal traces, and the other 5 are stamen traces. There are no well-defined traces to the missing petals. The ventral carpel traces depart from the stele at approximately the same level as the perianth traces (Figs. 43, 46). Midway between the base of the receptacle and the base of the locules the carpel dorsals branch off, 2 from the sepal traces and 1 from a stamen trace. The carpel dorsals move up the walls of the ovulary, curve over the top for a short distance, and then fade out. The ventrals after giving off short placentals pass up the style for some distance and then fade out (Fig. 42). The disk is not prominent in this species, and there is no evidence of vascular supply. The ovulary is partially immersed in tissue which is made



FIGS. 42–47. *Colletia crenata* Clos. FIGS. 42–43. Longitudinal sections through flower. × 11. FIG. 42. In plane of the style. 1, Edge of anther; 2, Style; 3, Carpel ventrals branching in style; 4, Disk. FIG. 43. Just off plane of style. 1, Anther edges; 2, Sepal; 3, Disk; 4, Ovule; 5, Carpel ventral; 6, Carpel dorsal. FIGS. 44–47. Cross sections through flower. FIG. 44. Through region of style. 1, Fused stamen-sepal laterals; 2, Style with distinct stylar canals; 3, Reflexed end of sepal; 4, Sepal laterals moving into sepal. × 11. FIG. 45. Midway up the ovulary, slightly oblique. Note incomplete concrescence of carpels. 1, Carpel ventral; 2, Mid sepal trace; 3, Carpel dorsal; 4, Stamen trace with a sepal lateral departing; 5, Carpel lateral; 6, Disk. × 11. FIG. 46. Through receptacle, somewhat oblique. 1, Carpel ventral; 2, Mid-sepal trace; 3, Fused stamen-sepal lateral. × 11. FIG. 47. Through the pedicel before departure of floral traces. × 41.

FIGS. 48–54. *Gouania conzattii* Greenm. FIG. 48. Cross section through style. 1, Carpel dorsal; 2, Carpel ventral; 3, Stylar canal; 4, Inner edge of disk. × 41. FIG. 49–53. Cross sections through flower. FIG. 49. Above the ovulary, slightly oblique, dotted line indicating approximate line between disk and perianth. 1, Part of petal blade; 2, Claw of petal; 3 Stamen trace; 4, Sepal; 5, Disk; 6, Style. × 11. FIG. 50. At level where style is just becoming

up of the walls of the ovulary, tissue of the disk, and tissue of the tube. At a point about mid-way up the ovulary the disk and perianth tube have become free from the ovulary (Figs. 43, 45). The whorls of the perianth remain fused as an urn-shaped structure to a point somewhat above the end of the style (Fig. 44). It is just above this point that the sepals become distinct and bend outward and downward. From the point at which the mid-sepal traces become distinct from the stele until the sepals become distinct, there are no major branches given off. The stamen trace does not branch off until just below the point at which the sepals become free. Here the sepal laterals split off radially from the stamen trace and one passes into the lateral margins of each sepal (Fig. 44). There is no clear evidence of the presence of vestigial traces to the missing petals. There is, however, a larger mass of tissue surrounding the stamen traces. In addition, the stamen traces are larger than the sepal traces (Figs. 44, 45). The larger size of the stamen trace might be accounted for by the fact that 2 sepal laterals are fused for most of their length with the stamen trace. At about the mid-point of the ovulary, there is a distinct ventral bundle in each carpel. Carpel dorsals and laterals are present, but they are not always distinct and readily recognized as in other genera. In the material studied there was apparently an incomplete concrescence of the carpels (Fig. 45). The 3 locules fade out at the top of the ovulary into 3 stylar canals and these are distinct to the top of the style (Fig. 44).

F. *GOUANIA* Jacq.

The flowers of *Gouania* are small, greenish white, 5-merous, and polygamous. The limbs of the clawed petals are usually scoop-like and each surrounds or encloses an anther. The margin of the disk is divided into 5 triangular lobes which are opposite the sepals and extend up along the sepals for some distance. The disk completely fills the tube and covers the 3-loculed ovulary. In some of the flowers examined there were 3 distinct styles, while in others the style was only 3-lobed. The capsule-like drupe, with 3 wings, splits at maturity into 3 carpels.

There are about 66 species of *Gouania* widely distributed in the tropics and sub-tropics.

G. conzattii Greenm.—The stele in the pedicel is highly dissected and roughly circular in outline (Fig. 53). Nine well-defined traces move outward from the stele in a very short vertical distance and a tenth trace separates laterally from one of the 9 at a slightly higher level. These traces are distinct at about the level of the base of the locules (Fig. 55). The petal-stamen and sepal traces

free, somewhat oblique. 1, Perianth tube; 2, Inner edge of disk; 3, Sepal laterals departing from the stamen-petal trace. × 11. FIG. 51. About mid way up the ovulary. 1, Petal-stamen trace; 2, Carpel laterals; 3, Petal-stamen trace; 4, Carpel dorsal. × 11. FIG. 52. Base of locules. 1, Mid sepal trace; 2, Ovule; 3, Carpel ventrals; 4, Mid sepal trace and stamen-petal-sepal lateral just after separation. × 41. FIG. 53. Through stele before departure of traces to flower. × 41. FIG. 54. Diagram of longitudinal section of flower, the left half in radial plane through petal mid rib, the right half through a sepal mid rib. 1, Petal; 2, Stamen; 3, Style; 4, Disk; 5, Edge of disk extending up sepal; 6, Sepal; 7, Carpel dorsal; 8, Trace to ovule.

which were fused at a lower level in the receptacle, i.e. the tenth trace to split off, stand near each other at this level. The carpel ventrals continue on upward after the perianth traces separate (Fig. 54). At the base of the locules they reach their normal position in the carpels and at this level the ventrals of adjacent carpels are distinct (Fig. 52). Here the carpels have not become completely concrescent (Fig. 52). At the level of the base of the locules there is still no evidence of the carpel dorsals (Fig. 54).

Before the level of the middle of the ovulary is reached, the carpel dorsals move out—2 from petal-stamen traces and 1 from a sepal trace. As they move up into the wall of the ovulary, a number of small lateral traces come off which move up into the wall of the ovulary and disk where they fade out (Figs. 50 and 51). Next, the perianth traces begin to move laterally and, bending, move up into tissue which will become sepals (Fig. 51). The carpel ventrals at this level are still distinct and the carpels themselves are still incompletely fused. There is some lateral branching of the vascular traces to the ovulary wall. At this level no distinction can be made between the disk, ovulary wall, and perianth members.

There is no separation of the disk from the ovulary. It is only when the style becomes distinct that the inner limits of the disk can be distinguished (Fig. 50). In the style there are 3 well-defined stylar canals each surrounded by 3 prominent vascular bundles (Figs. 48 and 50). Two of these bundles are branches of the carpel ventrals and the other one is the carpel dorsal which bent around the top of the ovulary and then turned upward in the style to parallel the ventrals (Fig. 54). Just before the dorsals enter the style the carpel laterals move across the top of the ovulary to a position almost touching the ventrals and fade out. Each of the 3 stylar canals with the 3 bundles around it continues on almost to the top of the style. While the pistil becomes free from the disk at this level there is still no distinction to be made between the disk and the various perianth members (Figs. 51, 54).

The ovulary is completely immersed in the disk. There is a thickening of the disk near the style which forms a ring about the style. The outer limits of the disk fuse with the base of the sepals and extend as a point up, but free from, the mid-line of the sepals (Fig. 54). In a cross section a short distance up the style all of the disk has disappeared except a ring which is fused with the bases of the petals and sepals (Fig. 49). The petals become free from the disk before the stamens do. The stamens arise directly from the tissue of the disk (Fig. 49). Above the level of the top of the locules the sepal traces give off a fairly large trace which moves across the top of the ovulary and sends branches into the disk. Well above the top of the ovulary smaller traces move from the sepal traces into the tissue of the disk (Fig. 54).

V. Discussion

Berkeley (1953) has pointed out the lack of consistency in the use of the term disk by various workers. "Thus we find anatomical studies resulting in both axial and appendicular interpretations of the origin of the disc, not only in different

families, by different writers, but even by the same writers in a single family. It seems quite reasonable to suppose that the disc is not a floral organ *sui generis*, but rather a *quasi* organ or structure incidental to floral development in some forms."

In this study of the rhamnaceous flower, however, there seems to be sufficient evidence to support the conclusion that the disk is of appendicular origin. The ovulary of *Rhamnus* is superior, being surrounded by a cup which is interpreted as being formed by the fusion of the bases of sepals, petals and stamens and is therefore a floral tube. This interpretation is based on the fact that the vascular traces to these structures are variously fused. Lining this cup is the disk. There are well-defined traces to the tissue of this disk which are fused with the perianth traces in the manner of the stamen traces. Carpel traces are fused with the petal-stamen and sepal traces for some distance. Since the carpel traces and the perianth traces leave the stele at the same level, i.e. just below the base of the ovulary it is concluded that the stele must end at this point.

The situation in *Berchemia* is somewhat like that in *Rhamnus*. The ovulary is superior, surrounded by, but not fused with, a ring-like disk. As in *Rhamnus*, the traces destined for carpels, stamens, petals, and sepals are fused to varying degrees. The sepals and petals are fused at the base but not to the extent they are in *Rhamnus*. There are smaller traces departing from the perianth traces much as the staminal traces do, and these move into the disk and end blindly. The carpel dorsals are fused with the petal-stamen traces and move out with them some distance before splitting off. The remaining stelar tissue soon becomes the carpel ventrals. Since the stelar tissue becomes the ventral carpels slightly above the point of departure of the fused perianth and dorsal carpellary traces, it is concluded that the stele in *Berchemia* ends at this point. It seems that the disk here may consist largely of the fused bases of the missing whorl of stamens.

The ovulary in *Colletia crenata* is semi-inferior, being adnate about half-way up the fused bases of the sepals. There are no petals in *C. crenata*. The disk is inconspicuous and is limited to the bottom of the floral tube. The fusion of the carpellary traces to the perianth traces presents about the same picture as that in *Berchemia* and *Rhamnus*. From the material available for study in this genus there is no evidence of any vascular tissue in the disk.

In *Zizyphus* and *Ceanothus* the ovulary is also semi-inferior. The tissue of the disk is intrusive tetween the fused bases of the sepals, petals, stamens, and the ovulary. In both *Zizyphus jujuba* and *Ceanothus americanus* the carpel dorsals are fused with the perianth traces to about the same degree and move out through the receptacle with them. The carpel ventrals continue on up from the top of the stele to supply the placentae. In *Zizyphus* there are many well-defined smaller traces leaving the perianth traces and moving up into the disk to end blindly. No vascular traces to the disk could be found in any of the material of *Ceanothus* examined.

Gouania conzattii is the only species examined in this study which has a completely inferior ovulary. Here again the picture of the adnation of floral whorls

and the fusion of perianth traces is similar to the condition found in the other species studied. The carpel dorsals, however, are fused for a greater distance than in the other species. There are distinct traces departing from the perianth traces in the plane of the staminal traces and ending in the disk. The disk tissue extends up the sepals for some distance, a condition which is peculiar to this genus.

It was pointed out earlier in this paper that in most of the family the sepals are covered interiorly by a fleshy and glossy layer which ends as a fleshy tubercle toward the extremity of the sepals. This layer is particularly evident in *Zizyphus* and in *Gouania*. Brongniart (1827) reports that he observed in *Gouania*, in at least one instance, anthers produced at the end of this fleshy layer and from this he concluded that the tissue of the disk represented tissue of missing stamens. Of particular interest in this connection are variations in the flowers of *Zizyphus* in one type of which the stamen is opposite the sepal.

While Bancroft (1935) points out that opinion is divided as to the value of using abnormally-developed flowers in morphological interpretations, this investigator is of the opinion that evidence from vascular anatomy here presented, together with the observations of the flowers of *Zizyphus*, supports in part the claim made by Brongniart that the thick fleshy part of the disk which lines the sepals represents a lost whorl of stamens. It would appear from the above evidence that the disk itself may be composed not only of the remains of another whorl of stamens, but also of tissue consisting of the fused basal portion of sepals, petals, and the existing stamens, and thus of appendicular origin. No evidence was found to support a receptacular origin.

A second question raised by this study will be considered now. Is this a family in which the evolution of a superior to an inferior ovulary is taking place? *Rhamnus* is considered to be the most primitive of the six genera studied and *Gouania* the most advanced. A number of considerations support this conclusion. In Table II the various factors used in attempting to arrive at the phylogenetic position of the six genera of this study are tabulated. A weight of 1 was assigned the primitive condition grading up to a weight of 5 for the advanced condition. *Rhamnus*, as shown earlier, has an almost world wide distribution. The ovulary is superior, adnation not having involved the gynoecium. One flower was observed with six sepals, petals, and stamens. While this variation is recognized as a very slight indication of primitiveness, expecially in view of the fact that much more variation was noted in *Zizyphus*, it is felt that it should be mentioned here, for the record, if for no other reason. The ovulary of *Rhamnus* is trilocular and tricarpellate. The same situation prevails in *Ceanothus*, *Colletia*, and *Gouania*. In *Zizyphus* and *Berchemia* there are, on the other hand, only two locules and two carpels. It might be noted that in these two genera the ovulary is semi-inferior. One may visualize how the flower of *Gouania*, considered here to be the most advanced, developed from the flower of *Rhamnus* by shortening of the floral axis with a resulting adnation and cohesion of the bases of the floral members.

It is further noted that in *Rhamnus* there has been no fusion of perianth traces with each other, except for the sepal laterals with the petal-stamen traces. In

TABLE II

Characteristics used in phylogenetic placement of genera studied

	Rhamnus	Berchemia	Zizyphus	Ceanothus	Colletia	Gouania
Ovulary	(1) Superior	(1) Superior	(3) Semi-inferior	(3) Semi-inferior	(3) Semi-inferior	(5) Inferior
Var. in fl. parts	(2) Little	(1) None	(3) Some	(1) None	(1) None	(1) None
Distribution	(1) Worldwide	(2) Widely scatter.	(3) Trop. & subtrop.	(4) N. Am. only	(4) S. Am. only	(3) Trop. & subtrop.
Number of carpels	(2) 3	(3) 2	(3) 2	(2) 3	(2) 3	(2) 3
Vascular fusion	(1) Little	(2) Below base of ovulary	(3) To top of ovulary	(4) High	(1) Little	(3) High
Fusion of perianth whorls	(2) Sep. & pet. at base	(2) Sep. at base, pet. none	(2) Sep. at base, pet. none	(2) Sep. at base, pet. none	(3) Sepals most of length	(2) All bases fused
Disk	(2) Lines bottom tube	(2) Bottom of floral tube	(3) Covers ovulary in part	(3) Partly covers ovulary	(3) Partly covers ovulary	(5) Covers ovulary

all other genera there is fusion to some degree. It may also be pointed out that there is reduction, if not entire elimination, of vestigial staminal traces as well as a great reduction of the disk as seen in *Colletia* and *Gouania*. Dawson (1936) found the same situation in Polemoniaceae and considered this to be an advanced characteristic. It seems to this writer that the evidence supports the conclusion that this is a family in which there is an evolution from the superior ovulary of *Rhamnus* to the inferior ovulary of *Gouania*. This is being accomplished by a general shortening of the floral axis which may be reflected in the vascular anatomy.

The history of the taxonomic treatment of the family by older authors suggests something less than full understanding of the position of the family as well as its intergeneric relationships. Adanson (1763) was the first to delimit the family under the name *Jujubiers* in which he included many genera since placed in in Rosaceae, Aquifoliaceae, and Celastraceae. A. L. de Jussieu (1789) divided the family into four sections each of which later became distinct families. Working from de Jussieu's concept of the family, Robert Brown (1814), divided the family into two families, the first two sections of de Jussieu becoming Celastraceae and the last two Rhamnaceae. Adolphe T. Brongniart (1827) separated the group into three families, Rhamnaceae, Celastraceae, and Ilicineae. Bentham and Hooker (1862–67) established five tribes: Ventilagineae, Zizypheae, Rhamneae, Colletieae, and Gouanieae. Except for the addition of the tribe Maesopsideae by Weberbauer in Engler and Prantl (1895), all subsequent works, including the latest, Suessenguth (1953), recognizes the same tribes as did Bentham and Hooker. Suessenguth places 58 genera and 984 species in Rhamnaceae. The tribes Rhamneae and Zizypheae are separated from the other three tribes by the fact that their fruits do not possess longitudinal wings, but rather are berries or drupes. Colletieae are separated from Gouanieae by the fact that the fruit of the Colletieae is dry and dehiscent and without wings, while the fruit of the Gouanieae has wings running lengthwise around it. It will be noted that the disk is not utilized at all in this tribal separation. It is concluded from the present study that the disk has only limited value in the taxonomic treatment of the family.

The pattern of fusion of carpel dorsals with petal and sepal traces is fairly uniform. In *Zizyphus*, with two carpels, the dorsals fuse with sepal traces; in *Berchemia*, also with two carpels, the dorsals are fused with the petal-stamen traces. In *Rhamnus*, *Ceanothus*, and *Colletia*, each with three carpels, two dorsals are fused with the sepal traces while the third dorsal is fused with the petal or petal-stamen trace. In *Gouania*, also with three carpels, the pattern is just reversed, i.e. one dorsal is fused with a sepal trace while the other two are fused with the petal-stamen traces. The explanation of these fusions can be found in the spatial relationships of the various whorls. If the hypothetical primitive flower is considered to be pentacyclic, then the first whorl of stamens would be in the same radial plane as the sepals and the second whorl would be in the same radial plane as the petals, i.e opposite them. In Rhamnaceae there is only one whorl of stamens and they are opposite the

petals. This is interpreted as indicating the loss of the first whorl of stamens. The hypothetical primitive flower may have had five carpels and, since each whorl alternates with the next one, that would mean that the carpels would be in the radial plane of the sepals. Now if shortening of the floral axis occurred and adnation of whorls took place, it is to be expected that fusion of vascular traces would follow. This would explain the fusion of the carpel traces with the sepal traces, as has been observed in *Rhamnus*, *Ceanothus*, and *Colletia*. If reduction of the carpel number from five to three occurred, as is the case here, a problem presents itself in the positioning of a three-sided structure inside a five-sided one. It is impossible to place a three-sided structure inside a five-sided one in a position so that each side of the inner figure is in the plane of a side of the five-sided one—i.e. three carpels in the same radial plane as that of any three sepals. This seems to be the only explanation possible for the fusion of two carpel dorsals with sepal traces, while the third dorsal is fused with the petal-stamen trace. There is no problem involved in the case of two carpels. In the case of *Gouania*, however, the answer is not clear.

It has been pointed out by Masters (1877) that the alternate arrangement of floral parts is so uniform that any exceptions are of especial interest. In Rhamnaceae the inner whorl of stamens is present (the whorl opposite the petals) while the outer whorl (the whorl opposite the sepals) is absent. This condition is generally called by various authors obdiplostemony (Puri, 1951, Johnson, 1931). Puri suggests that the simplest way to explain this situation is that the outer antesepalous whorl just disappeared, as seems to be the case in certain Geraniaceae. In certain Rhamnaceae this whorl of stamens is represented by the traces in the same radii as the mid-sepal traces which move up into the disk to end blindly. A second situation is mentioned by Puri in which case "the antepetalous stamen bundles do not arise separately but are carried out conjoined with the petal midrib bundle and in this way, as has already been suggested, may be brought to a position farther from the center than that reached by the independently arising antesepalous stamen bundle." This seems also to be the state of affairs in Rhamnaceae. Arber (1939) interpreted this superposition of stamens on petals to "form a unit in whorl alternation without being a unit in the morphological sense." With this interpretation the present writer agrees.

The peculiar behavior of the carpel ventrals in *Zizyphus* was pointed out earlier. De Candolle's (1825) classical interpretation of the carpel regards that structure as a leaf-like organ folded upward (ventrally), the margins more or less fused and bearing ovules. Further evidence of the folded nature of the carpel is the fact that in most instances the carpel ventrals are inversely oriented. Henslow (1890), on the other hand, reports a few examples of normally oriented carpel ventrals. It seems to this investigator that in *Zizyphus* (Figs. 12a–12i) the process of this inversion is seen taking place to a remarkable degree.

An interesting situation is present in the development of the ovules in Rhamnaceae. The various taxonomic treatments of the family describe the ovules as being solitary and basal. That they are basal in their placentation is clear. The ovules do, however, develop from the lateral margins of the carpels. In *Zizy-*

phus and *Berchemia* where the bicarpellary gynaeceum has only two ovules, one in each locule, the margin of only one of each carpel bears the ovule, the other margin being sterile. In *Zizyphus* where the ventrals to each carpel are not fused, the ovule gets its vascular supply from the ventral of the margin on which it develops. Since the ventrals are fused in *Berchemia* it cannot be said that the same situation as to vascular supply holds true in that genus. Puri (1939) in his work on *Garcinia* reports a similar occurrence. In the tricarpellary, trilocular gynaecia of *Rhamnus*, *Ceanothus*, and *Gouania* the same general situation is noted, but with some variation as to which margin is fertile. In *Rhamnus* the order is as follows: fertile-sterile, fertile-sterile, and sterile-fertile. In *Ceanothus* the order is: fertile-sterile, fertile-sterile, and fertile-sterile. In *Gouania* the order is: sterile-fertile, sterile-fertile, and fertile-sterile. Suessenguth (1953) reports somewhat the same condition and presents a reduction series attempting to show how the unilocular condition of *Maesopsis* could have developed from the 4-locular *Rhamnus*.

As was mentioned earlier, Rhamnaceae and Celastraceae are placed close together in many systems of classification. It was hoped at the beginning of this research that evidence would be found which, together with the details of the floral morphology worked out by Berkeley in Celastraceae, would shed some light on the relationships of these two families. While the present investigation has been rather broad, i.e. dealt with one or two genera from the major tribes rather than many genera from a few tribes, there are a few trends apparent so far as relationships with Celastraceae are concerned. More complete studies on both families should be made; studies which will cover not only floral morphology, but also characteristics of the wood, distribution, serological characteristics, etc. before reliable conclusions as to phylogenetic relationships can be reached. It seems, however, from the evidence at hand that the most likely bridge between the two families is from *Celastrus* to *Rhamnus*. Both have tri-locular ovularies standing in, but free from, a cup of other tissue. Each also has five sepals, five petals, and five stamens, but instances have been noted in both where these parts vary. The vascular supply to floral parts of the two genera is very similar. The big difference between the two is, of course, the occurrence of the stamens opposite the petals in *Rhamnus* while in *Celastrus* they are opposite the sepals. One could visualize a hypothetical common ancestor of these two genera in which there were two whorls of stamens. By a loss of the outer whorl *Rhamnus* would have resulted while the loss of the inner whorl would have produced *Celastrus*.

SUMMARY

The vascular anatomy is described for one species of *Zizyphus*, one of *Berchemia*, two of *Rhamnus*, six of *Ceanothus*, one of *Colletia*, and one of *Gouania*.

It is shown that *Rhamnus*, the most primitive of those studied, has a superior ovulary which is surrounded by, but not adnate to, a disk. The situation is essentially the same in *Berchemia*. In *Colletia* the ovulary is gradually sinking into the disk with the disk becoming inconspicuous. In *Zizyphus* and *Ceanothus*

the ovulary is also partially immersed in the disk, but the disk is more prominent and intrusive between the bases of the perianth members. *Gouania* is the only genus studied which has a completely inferior ovulary. Further evidence is present to support the conclusion that the disk represents a missing whorl of stamens as well as tissue resulting from the fusion of perianth whorls. It is further concluded that this is a family in which there is a gradual transition from a completely superior ovulary to a completely inferior one.

There seems to be no basis for reworking the taxonomy of Rhamnaceae on the basis of the expression of the disk. Tribal lines, as presently set up on the basis of fruit characters, are cut across quite freely by the expression of the disk. (Table I). It is the opinion of the writer that fruit characters are more reliable in showing relationships than is the disk which might vary according to spatial pressures.

It is noted that this family is another of the obdiplostemonous families and that the superposition of the stamens and petals is interpreted as a unit in whorl alternation without being a unit in the morphological sense.

It is suggested that the family Celastraceae may be linked with Rhamnaceae through a hypothetical common ancestor which had two whorls of stamens. A loss of the antesepalous whorl gave rise to Rhamnaceae while loss of the antepetalous whorl produced Celastraceae.

The behavior of the carpel ventrals of *Zizyphus* is described and interpreted as showing steps in the inversion of the ventrals during their passage from stele to ovulary.

Finally, the ovules which are basal are shown to develop from carpel margins. The situation as to fertility of carpel margins varies considerably.

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NORTH AMERICAN LEJEUNEACEAE

I. INTRODUCTION; KEYS TO SUBFAMILIES AND GENERA[1]

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The members of the family Lejeuneaceae are distinctive plants, of tropical derivation, which occur in the United States largely in the extreme southeast. In the most recent lists (Buch, Evans, Verdoorn, 1937; Evans, 1940) 53 and 55 species, respectively, are listed from the United States, assigned to twenty-two genera. The writer (Schuster, 1954) was able to add one additional genus and

[1] Succeeding papers in this series will each deal with isolated genera or groups of genera. The second paper in this series, II. Paradoxae: The Genera *Aphanolejeunea* and *Leptocolea* follows immediately after this paper.

All bibliographic citations will appear in the final number of this series of papers.

species to this number. In a separate contribution (Steere and Schuster, 1955) still another species and genus are added to our flora, while in the immediately succeeding sections of this series, two additional species of *Lejeunea*, and two new species of *Aphanolejeunea*, are recorded from our region. Fulford (1945) first reported *Ceratolejeunea rubiginosa* from our area. The total number of species treated in this series of papers, therefore, includes 62 species, making the Lejeuneaceae much the largest family of Hepaticae of our region. Of these species, one has recently been described from the Arctic Coast of Alaska (Steere and Schuster, 1955), and enjoys the distinction of being the only species of an otherwise largely tropical family limited to essentially the area north of the Arctic Circle. A second species, *Cololejeunea macounii*, occurs with an equally restricted range in British Columbia, and has been found only twice. The other species fall into two categories, a small group of essentially Appalachian distribution (*Lejeunea ruthii*, *L. ulicina*, *L. cavifolia*, *L. patens*, *Drepanolejeunea* sp., *Leucolejeunea clypeata*, *Cololejeunea biddlecomiae*), and a much larger group of species restricted largely or entirely to the Coastal Plain of the southeastern United States. A few of the members of this last group (*Leucolejeunea conchifolia*, *L. unciloba*, *Cololejeunea ornata*, *C. minutissima*, *Rectolejeunea maxonii*, *Crossotolejeunea bermudiana*, *Lejeunea glaucescens* and *L. laetevirens*) also occur, more or less sporadically, in or near the escarpment of the Appalachian plateau, evidently as Tertiary Relicts. At least twenty-four of the species are confined, within our area, to Florida.

Previous work on the North American Lejeuneaceae has been almost exclusively that of Dr. A. W. Evans, who treated most of our species, at one time or another, in a long series of papers admirable both in their accuracy and richness of detail, and in their precise, detailed illustrations (Evans, 1902, 1903, 1903a, 1905, 1906, 1906a, 1907, 1907a, 1907b, 1908, 1908a, 1908c, 1911, 1911a, 1912, 1917, 1918, 1938, 1940). In addition, Evans (1902) had published the only preceding revision of the North American members of this family.[2] In that revision, only 23 species were recorded from the region. From this it is evident that the species of Lejeuneaceae known from North America (north of Mexico) have been increased almost three-fold in the last half century, thus necessitating a total revision of the members of the family.

Recent work on the Lejeuneaceae has been extremely limited. Fulford (1945) published a revision of some of the species of *Ceratolejeunea*, including the three regional species. Evans (1935) published an important study on the stem anatomy. More recently Schuster and Hattori (1954) have published a study of the extra-nuclear cytological features of the members of the family, including a treatment of almost all the North American species. Schuster (1954) gave revised keys to the Holostipae, and extensions of range of the genera *Ptychocoleus*,

[2] This paper remains of considerable importance because of the detailed discussion of the early history of regional members of the family, and the careful derivation of the synonymy of our species. For the earlier historical portrayal of work on this family, this paper should be consulted.

Lopholejeunea and *Neurolejeunea* to new northern limital points. The only attempt at a general treatment of the family is in Frye and Clark (1947). In this work, many of the fine illustrations of Evans have been redrawn, with loss of much significant detail and of accuracy. The descriptions and distribution data of Evans have also been compiled. Insofar as the work leans directly on the accurate revisionary papers of Evans, it is valid and useful, even if it does not further advance our knowledge. Unfortunately, a very considerable number of errors have been introduced, largely in the keys and the generic diagnoses. For the non-specialist, their use can only lead to a high percentage of misdeterminations, reflected in the high level of erroneously identified material which has come to the writer's attention in recent years. Another inevitable result has been the compilation in recent years of "local lists" including range extensions hardly credible to the more sophisticated worker. The need for a major revision of the family is thus acute for two reasons: no critical revision has been published since that of Evans (1902), and the recent work of Frye and Clark (1947) includes so many errors that it can only lead to a further erroneous literature, on this family, if blindly followed.[3]

The present revision is based on personal acquaintance, in the field, with all but six of the 62 species at present known from our area; these six species have been studied from herbarium material. Field work, emphasizing the microenvironmental distribution of these exacting plants, has been carried on extensively for five years, with experience with a limited number of species in previous years. Seven collecting trips (averaging 2–3 weeks in extent) have been made to all parts of Florida; in addition, over 12 long field trips have been taken into the Southern Appalachian escarpment, and to the Atlantic Coastal Plain. The following revisionary data are, consequently, based to a high degree on personally collected, living materials.

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The writer is indebted to the New York Botanical Garden (and specifically to Dr. Donald P. Rogers and Miss Rosalie Weikert) for many courtesies and for the loan of material; the New York Botanical Garden also provided research space (summer 1952, summer 1954) and a grant for research for two months in 1954. Much of the field work serving as background for this series of papers has been made possible by a National Science Foundation Grant (NSF Grant 669), which has also made possible the numerous illustrations, and defrayed the cost of secretarial help. To Drs. H. L. Blomquist and L. E. Anderson the writer is indebted for a critical reading of portions of this series of papers. My wife, Olga M. Schuster, helped throughout the preparation of this manuscript, in the assembly of figures, and in the collection of materials. In the collection of the epiphyllous, microscopic species, the help of my daughter, Erica Schuster, must be acknowledged; the acuity of vision of a child here has certain advantages.

[3] Originally, a section was devoted to a systematic correction of errors in this work; it appears impossible to justify the space this would entail. Consequently, only the most serious errors are noted (usually in footnotes) on succeeding pages.

FAMILY LEJEUNEACEAE

Plants *usually yellow-green* but sometimes with brown pigmentation, decumbent or prostrate, *bearing only lateral branches; branches usually of Radula-type* (i.e. from basiscopic portion of ventral half of lateral merophytes), rarely of *Frullania*-type (and then from acroscopic half of lateral merophytes, replacing the ventral portion of the leaf). Rhizoids restricted to small regions *at bases of underleaves* (or to the points at which these have been suppressed). Leaves unequally bilobed, the much larger dorsal lobe entire, more rarely dentate, never lobed, *incubously inserted and oriented*; ventral lobe (*lobule*) folded under lobe, *inserted by a line that arches slightly to distinctly towards the stem apex*, as a consequence distinctly attached to the stem as well as to dorsal lobe, more or less strongly *inflated and forming a sac lying roughly parallel to posterior margin of lobe; keel between lobe and lobule elongate*, the lobule attached by almost its entire length to lobe; apex of lobule marked usually by a small tooth, at or near whose apex is situated a hyaline papilla; proximal base of lobule, at point of insertion on stem, with a hyaline papilla which is often stalked or situated on a filiform row of cells, the stylus. *Underleaves almost always present*, 2–0-lobed, absent only in taxa with reduced ventral merophytes. Cells essentially collenchymatous, *always with oil-bodies, often dimorphic* (with ocelli). Asexual reproduction fundamentally of three types (a) by caducous leaves, which may or may not be modified in form and size; (b) by short, small-leaved, lateral branches which are caducous; (c) by discoid brood-bodies, formed on the margins, lower or upper surfaces of the lobes; "true" or fasciculate, few-celled *gemmae never developed*.

Antheridia spherical, *longly pedicellate, the stalk uniseriate*, usually 1 or 2 per bract; body of antheridium formed fundamentally of 2 tiers of elongate cells, several small basal cells, and several apical, small cells. *Archegonia only 1 per inflorescence*; the *archegonium very long-necked*, the neck projecting beyond the mouth of the perianth until the sporophyte develops. Perianth extremely polymorphic, typically obovoid and with 5 longitudinal carinae (one dorsal, 2 lateral, 2 postical), of which the dorsal is often absent, and the two postical united to form an obtuse postical keel; often with development of several to many supernumerary longitudinal carinae; *perianth narrowed* at apex *to a usually well-marked slender beak. Frequently with development of innovations from below the female inflorescence*, which always orignate directly below the bracts; bracts always in a single pair.

Sporophyte *short-stalked*, the *seta of 2 cell-layers*, the inner usually of 4, the outer of 12 or 16 cell-rows; *cells of seta in regular, even tiers*, thus at maturity distinctly articulated in appearance. Capsule spherical, 4-valved for three-fourths its length, the *walls always bistratose*; walls without sharply defined annular or semiannular bands, the thickenings irregular or often virtually absent, when present largely restricted to radial walls of corners of epidermal cells. *Elaters few*, 1-(rarely 2-)spiral, the spiral often fused with thickened elater-wall and becoming obsolete, *attached* by a narrow, constricted base *to the distal portions of the valves*, the apex dilated, thus *trumpet-shaped*. Spores *precociously several-celled* before opening of capsule, *large*.

The Lejeuneaceae, together with the closely related Frullaniaceae, represent one of the apices of liverwort evolution. They include the most specialized of the leafy Hepaticae, and, judging from the number of species and their wide distribution in tropical regions, one of the most successful groups among a relatively "unsuccessful" class. At present about 1600 species are known, placed in over 65 genera. However, the work of Verdoorn (1934) suggests that the group is strongly over-inflated, and that many of the species which have been described, particularly by Stephani, are ill-founded. Prolonged and critical work suggests that this also holds for a number of the genera. In spite of this, the Lejeuneaceae constitute the largest family of Hepaticae, and include, in tropical regions, the most ubiquitous of the liverworts.

The vast majority of the Lejeuneaceae are tropical and subtropical in distribution. Judging from the concentration of endemic genera and species, the northern half of South America appears to have served as a center of evolution of the group. In the region here covered, only a sparse representation of species and genera occurs, the majority restricted to peninsular Florida (in our area). Consequently the problems faced by the student of the group in the tropics are met with in only a diminished fashion in our region. For the student of the family, as represented in the United States, the classical series of papers on "The Hepaticae of Puerto Rico" (Bull. Torrey Bot. Club, 1900–1911) by A. W. Evans, is of fundamental importance, as is that author's study of the stem anatomy (Bull. Torrey Bot. Club, 1935). Vanden Berghen (1948) has recently compiled a key to the genera and a conspectus.

In the determination of members of this family, several specialized features are of much help. Among these are the following: (1) The position of the inflorescences, whether on short lateral or elongate lateral shoots. (2) Whether 1 or 2 subfloral innovations occur below the female inflorescences, or whether such innovations are absent. (3) The presence or absence of specialized cells, the so-called ocelli, each containing a large, conspicuous oil-body; Zwickel (1932, 1932a) has dealt with the distribution of the ocelli in detail, as have Schuster and Hattori (1954). (4) The size, form and number of oil-bodies per leaf-cell; Schuster and Hattori (1954) have dealt with the cytological aspects of the classification of the Lejeuneaceae. (5) Form of the Lobule. Evans, in his various papers, has correctly emphasized the fundamental importance of the form of the lobule and of the position of the hyaline papilla, in relation to the apical tooth of the lobule. In some cases, the hyaline papilla is situated beyond the apical tooth (e.g. in the sinus between apical tooth and the keel); in such cases it is considered to be *distal*, with respect to the apical tooth. In other cases, it occurs in a small emargination at the base of the apical tooth towards the stem (or base of the lobule), in such cases it is considered to be *proximal*. In a relatively few cases, the hyaline papilla has been displaced to the interior face of the lobule, away from the margin; in such cases it is spoken of as *ental* in position. There appears to be no doubt whatsoever that the position of the hyaline papilla is always correlated with characters of generic importance; i.e. any one genus always shows a uniform position of the hyaline papilla. As a consequence, the determination of sterile

material, which is always fraught with difficulty, must usually involve a study of the form of the apex of the lobule, and of the position of the hyaline papilla.[4]

RELATIONSHIP OF LEJEUNEACEAE

The Lejeuneaceae show distinct affinities with both the Frullaniaceae and the Porellaceae, but to no other families of Hepaticae. Although the gametophyte of the Lejeuneaceae normally appears very different from that of the Porellaceae, occasionally we find startling similarities. These are exhibited by *Porella platyphylloidea* var. *radulopsis* and such genera as *Lopholejeunea*. It is probable that the Lejeuneaceae and the Frullaniaceae represent a highly derivative offshoot of the Porellaceae.

The relationship to the Frullaniaceae is particularly close. This is especially the case with the sporophyte. In both families the epidermal cells of the seta are in tiers, giving the mature seta an articulated appearance; in both the capsule-wall is bistratose, with the large epidermal cells often bearing vertical (radial) thickenings of the corners (and sometimes elsewhere); in both we find a reduced number of elaters, trumpet-shaped, attached in a sharply restricted pattern to the distal positions of the valves; in both, the spores bear ring-like groups of papillae and precociously germinate, becoming multi-cellular within the unopened capsules.

However, the gametophytes exhibit important differences. The Lejeuneaceae are fundamentally yellowish-green to green plants, in which secondary pigments are brownish, never reddish; the Frullaniaceae exhibit a pattern of variation from pure green (when without secondary pigments) to reddish-brown or purplish and are almost never fuscous or yellow-green. In the Lejeuneaceae, with exceptional secondary modifications omitted, we find that the lobule is attached by a broad line to the lobe, resulting in a discrete keel, usually parallel to the postical margin of the lobe; furthermore, the lobule is attached to the axis by a more or less elongate, virtually longitudinal line of insertion; the lobule is strongly convex, with the convexity formed by what is morphologically the *abaxial* leaf surface; usually the anterior (free) margin of the lobule is involute and appressed to the lobe, while the distal margin (adjacent to the keel) is open, often forming a nearly circular opening to the otherwise fairly tightly closed water-sac.[5] By contrast, the Frullaniaceae show leaves that are divided virtually to the base, and the lobule is typically almost free from the leaf (being attached

[4] This is usually easily accomplished by tearing off, or cutting off several leaves, preferably young ones, and flattening these, by gentle pressure, between slide and cover-slip. Most frequently the lobule is so convex, in the undamaged plant, with the anterior or free margin so strongly involute, that the apical tooth and lobule are not readily apparent without such a procedure.

[5] Under exceptionally moist conditions this normal type of lobule may be variously modified. In some species, with normally convex and inflated lobules, the mesic extreme of the species shows explanate lobules, forming merely a flat fold. This is well illustrated by *Cololejeunea minutissima*. Similarly, some species restricted to very shaded and humid sites normally fail to show inflated lobules, the lobule then becoming a small, reduced fold. *Cololejeunea setiloba* is an example of a species showing constant reduction of the lobule.

to it by a very short line), and almost free from the stem, to which it is never connate for any distance. Furthermore, the lobule is inflated in a fundamentally different fashion, the lobule-margins becoming reflexed to the point where a helmet-shaped ("galeate") lobule results, with the convexity formed by the *adaxial* face of the leaf (the *abaxial* surface lining the inside surface of the lobule).[6] Correlated with this mode of origin of the lobule, we find that the opening to the lobule is directed towards the stem or towards the posterior leaf-margin, rather than towards the leaf-apex, as in the Lejeuneaceae.

An important distinction, unfortunately subject to much modification, is offered by the underleaves. These, in the Lejeuneaceae, are bifid, entire, or may be absent in reduced genera. However, when they are entire, they are inserted on relatively broad ventral merophytes, usually 4-more cells broad, whereas, when bilobed, they are almost invariably inserted on narrow merophytes, 2 cells broad; in those cases where the underleaves are lost, the ventral merophytes are almost always reduced to the width of a single cell. By contrast, the Frullaniaceae show, with very few (and no local) exceptions, bifid underleaves. These are always inserted on broad ventral merophytes, never reduced to a width of 2 cells. As a consequence, the members of the Lejeuneaceae with bilobed underleaves can be distinguished from the Frullaniaceae, with which they might be confused, and can be separated by the biseriate cortex of the ventral merophytes, that of the Frullaniaceae being always polyseriate.

The mode of branching, in addition, offers important, if not absolutely constant, means of separating the two families. In the Frullaniaceae we find that the branch develops from the whole of the ventral half of a lateral merophyte; as a consequence it always replaces the ventral half of a leaf, i.e. the lobule. Correlated with this we find that the first leaf of a branch is an underleaf, the second a lateral leaf oriented towards the apex, the third a leaf oriented away from the apex of the main shoot. This type of branching has been called by Evans (1912) the *Frullania*-type. It occurs uniformly throughout the Frullaniaceae and the Porellaceae. In the Lejeuneaceae we find that the more robust, pigmented, primitive Holostipae at times show this type of branching, as for instance in *Brachiolejeunea* and *Stictolejeunea*. However, in all but a few of the primitive genera we find that branching occurs almost without exception by a type called the *Radula*-type. In this type, the branch originates from the posterior (basiscopic) portion of the postical half of the lateral segments, thus not involving the portion of the segments from which the lobules arise. As a consequence, this type of branching invariably shows a branch arising *immediately posterior* to a well-developed leaf, with a normal lobule. Correlated with this, we find that the first leaf of a branch is an underleaf; the second a lateral leaf turned away from the apex of the parent shoot; the third a lateral leaf oriented towards the parent shoot. In addition to this, the normal pattern of branching, we find that

[6] As in the Lejeuneaceae, unusually moist environmental conditions may result in a failure of the lobules to undergo their normal development; in such cases, the lobule is a lanceolate or lingulate structure, lying parallel to and near the stem, with its margins and apex more or less reflexed, thus foreshadowing the normal lobule type.

in the Lejeuneaceae almost all of the genera show the production of a specialized type of branch, the *innovation*. The innovation is borne immediately behind one, or sometimes both, of the female bracts (which are reduced, in the Lejeuneaceae, to a single pair). The innovations are often small and of limited growth, but more frequently as large as the axis from which they arise. In the latter case, the inflorescence is usually displaced, superficially becoming lateral (really "pseudolateral"), while the innovation may appear to be a direct continuation of the main axis. When two innovations occur, and develop equally, the inflorescence becomes situated in what appears to be the crotch of a dichotomy. In either case, the single or the paired innovations are often soon again floriferous, often resulting in a characteristic and highly derivative arrangement of inflorescences ("flower-clusters"), as in some species of such genera as *Taxilejeunea*. By contrast, in the Frullaniaceae we never find true subfloral innovations, i.e. no branches ever develop immediately behind the perichaetial bracts. However, frequently branching occurs one or two leaf-pairs beneath the inflorescence, and the perianth may then also become pseudolateral by the vigorous growth of the innovation. In the Frullaniaceae we also find several pairs of subfloral leaves modified, and may properly speak of more than one pair of perichaetial bracts.

The distinctions drawn above between the two groups are unfortunately bridged, to some degree, by the genus *Jubula*. In this we find branching of the *Frullania*-type, the position and form of the lobule is as in *Frullania*, secondary pigments are not developed, the stylus (as in most Lejeuneaceae) is reduced to a hyaline papilla, while subfloral innovations (on both sides, or more rarely only on one), similar to those of the Lejeuneaceae occur. Evans (1902, p. 394) also calls attention to the fact that the androecial branches in *Jubula hutchinsiae* appear to arise by the *Lejeunea* Type (i.e. behind a normal, lobulate leaf), even though the Hawaiian *Jubula piligera* shows origin of the male branches by the *Frullania* Type. In the seta of the sporophyte, *Jubula* is identical with the Lejeuneaceae, (having the seta formed of a restricted number of cells, oriented in two concentric layers), while in *Frullania* the seta cells are numerous, arranged in four concentric layers. Although the trigonous, flattened perianth of *Jubula* closely matches that of many species of *Frullania*, similar perianths occur in some Lejeuneaceae.

In spite of the synthetic features exhibited by *Jubula*, the writer would agree with other recent students in according family status to the Lejeuneaceae. In the majority of their features (particularly the unique reduction of the female inflorescence to a single, extremely long-necked archegonium; the reduction of the articulated short seta to two concentric rings of cells; the highly modified leaves; the peculiar, sessile elaters) the Lejeuneaceae represent the most highly specialized family of leafy Hepaticae. As such, they have produced such exceedingly derivative types as *Metzgeriopsis*, in which the vegetative axes are reduced to flattened, costate, *Metzgeria*-like thalli, while only the sexual branches bear reduced leaves. The student of the North American flora can obtain a very limited idea of the extraordinary diversity and polymorphism of this, the largest family of Hepaticae.

EVOLUTION WITHIN THE FAMILY

The vast number of genera (66–70 at present, depending on individual preference), and the extraordinary polymorphism of some features (perianth-form; underleaves, etc.) make it very difficult to trace the phylogenetic history of the group. Spruce (1885, p. 74) first divided the family into two subgroups, the Schizostipae (with divided underleaves) and Holostipae (with entire underleaves). This division, though at first glance seemingly of slight importance, is of considerable significance, *if* we take for granted the fact that some Schizostipae (among them *Cyrtolejeunea*, *Byssolejeunea*, some species of the genera *Cyclolejeunea*, *Cheilolejeunea* and *Ceratolejeunea*, etc.) have entire underleaves, while some Holostipae have more or less emarginate ones (as, for instance, *Caudalejeunea*). It seems paradoxical to use, as a subfamily character, a feature which is not even constant in such single genera as *Cyclolejeunea*. The writer would be the first to admit that the underleaf form, as such, is of little significance, *but* it is correlated with considerably more significant features derived from the stem. In the Holostipae, with the exception of a very few types, such as *Anoplolejeunea*, the stem is relatively robust, and consists of 10–70 cortical cell-rows. On the other hand, in the Schizostipae, with the exception of *Potamolejeunea*, a genus of dubious relationships, we find that the stem consists of seven rows of cortical cells, and these are almost invariably much larger than the medullary cells. Correlated with this, we find that in the Schizostipae, the ventral merophytes (which produce the underleaves) are usually 4–10, rarely more, cells wide; in a few extreme cases, only three cells wide. On the other hand, the Schizostipae, with their seven rows of cortical cells, show the ventral merophytes uniformly two cell-rows wide. In the writer's opinion, this latter feature should be utilized as the critical feature in separating the two groups. It has the further very obvious advantage of being easily ascertained, even from a small section of a sterile stem, since the number of cell-rows on which the underleaves are *inserted* is easily determined.

The writer would not attempt to maintain that the Holostipae and Schizostipae, as defined, are monophyletic groups. Such genera as *Ceratolejeunea* are on the borderline. Furthermore, parallel reduction-series undoubtedly have occurred, e.g. from a holostipous form with robust stems, to a reduced type with the stems bearing seven cortical cell-rows (and with either entire or bifid underleaves). However, with our present state of knowledge, it appears scarcely profitable to attempt to arrange a polyphyletic system.

In 1908, Lacouture (p. 102) simplified the classification of the family very considerably by splitting off from the Schizostipae a third group, the "*Lejeunea paradaux*," which Evans (1935, p. 187) suggests calling the Paradoxae. This group is certainly a sharply definable and natural group, the most highly specialized of the family, derived evidently from the Schizostipae. We find in this group a reduced stem, with one or three medullary cell rows, and a maximum of seven cortical cell-rows, of which two form the ventral merophytes. Such a type differs from the Schizostipae in that the underleaves have become doubled on the vegetative axis. In consequence, there is now an underleaf for every lateral leaf. With even greater reduction, the stem is reduced within this group to five cortical

rows (of which a single ventral row forms the ventral merophytes; e.g. the ventral merophytes, as cut off from the apical cell, are not again divided by longitudinal walls) and a single medullary cell-row. Associated with this extreme reduction we have a situation where the single row of ventral cells no longer can produce discrete underleaves (at best only hyaline papillae that disappear with age), but where the arrangement of a rhizoid-initial (or rhizoid-initial group) associated with each lateral leaf still clearly indicates the Paradoxae-type of segment-ontogeny.[7]

KEY TO SUBFAMILIES OF LEJEUNEACEAE[8]

1. Ventral merophytes 1–2 cells broad (the underleaves, if present, inserted on 2 cell-rows); underleaves bilobed for ¼ or more of their length, or absent; perianths fundamentally 5-carinate (but often with the dorsal carina obsolete or absent, and sometimes the 2 postical carinae united to form a single keel; never developing supplementary carinae; rarely with carinae all obsolete); plants almost always delicate, usually green or yellowish-green (rarely with golden to brownish pigmentation); stem anatomy simple: the cortical cells in 5–7 rows; frequently with asexual modes of reproduction ..... 2.
   2. Underleaves always present, occurring one per each pair of lateral leaves, inserted on 2 cell-rows; rhizoid-initial regions one per leaf-pair; lobules with a single apical tooth. ..... SCHIZOSTIPAE
   2. Underleaves absent or present, if present one per lateral leaf; rhizoid-initial groups always one beneath every lateral leaf; ventral merophytes 1–2 cells broad; lobules with an apical tooth and a second, proximal tooth, separated from it by a distinct sinus. PARADOXAE
1. Ventral merophytes 4 or more cells broad (the underleaves present, inserted on 4 or more rows of cells); underleaves entire or at most emarginate; perianths usually fundamentally 3-carinate (but often with several to many supplementary carinae, then 5–12-carinate); plants robust, usually brown or fuscous pigmented; stem anatomy complex, the cortical cells usually in 10–35 cell rows; rarely with asexual reproduction ..... HOLOSTIPAE

SUBFAMILY HOLOSTIPAE (Spruce, 1885)

Subtribe Atomiae Frye and Clark, Univ. Wash. Publ. Biol. **6:** 893, 1947.

Rather large to very large plants (usually 800–2500 μ wide), *almost always showing pronounced* yellowish to brownish, rarely reddish, secondary *pigmentation.* Stems robust, the *cortical cells usually in 12–36 rows,*[9] the medullary cells in numerous rows; branching by the *Radula*-type (and sometimes also by the *Frullania*-type); ventral merophytes 4-several cells broad, normal in number (i.e. one ventral merophyte for each pair of lateral merophytes), with conse-

[7] Frye and Clark (1947) have separated this reduction-series into two (Aphylliae, Diplasiae), which is here regarded as quite unnecessary. On this basis, the Subgenus *Leiocolea* of *Lophozia* could be split into two series also. These writers have furthermore adopted new names for the other two groups (Atomiae for Holostipae; Tomiae for Schizostipae), which the writer regards are totally without any valid basis. He can see, furthermore, no advantage in changing to these newer names.

[8] The Keys to Genera are given under the individual subfamilies.

[9] Evans (1935) has shown that *Anoplolejeunea*, a monotypic genus of tropical America, has only 7 rows of cortical cells, exactly as in the Schizostipae. This also has the sharply 5-carinate perianth of that group, and may, on further study, be better placed in the Schizostipae, in spite of the entire underleaves. The total lack of pigmentation of the cell-walls also suggests that such a disposition may be warranted.

quently one underleaf per pair of lateral leaves developed. Leaves with lobes inserted by a discrete, oblique, incubous line, the insertion acroscopically arching ventrally and extending for some distance up the stem (lobule consequently broadly attached to stem); lobes often variously dentate; lobules sometimes with teeth of the free margin, the hyaline papilla various in position. Cells almost always strongly collenchymatous, usually with secondary pigmentation; ocelli rarely developed (never in ours); oil-bodies very diverse, ranging from minute and homogeneous, many per cell, to very large, segmented, 1 per cell. *Underleaves large, essentially unlobed or at best retuse at apex.* Asexual reproduction rarely present (by caducous leaves in some species of *Ptychocoleus*, by discoid gemmae of the antical leaf-surface in *Caudalejeunea*). Perianths fundamentally 3-carinate, often developing supplementary carinae (then 6–12-plicate), rarely 5-carinate (*Leucolejeunea*, *Anoplolejeunea*, etc.); bracteole large, usually entire. Sporophyte with seta (as far as known) formed of 4 central rows of cells surrounded by a cylinder of 16 cell-rows (in *Leucolejeunea* only 12 rows).

The Holostipae include a moderate number of genera (23 or 24), most of which are relatively sharply circumscribed. The group includes the most robust, often the most highly pinnatifid of the Lejeuneaceae. Associated with the robustness, growth is frequently away from the substrate, by means of a secondary stem system (and occasional formation of festoons), a xeromorphic structure (thick-walled cells of the stem; strongly collenchymatous leaf-cells), and most often a development of pronounced secondary pigmentation. On the basis of the latter, the species of the present group may be confused with the Frullaniaceae. However, unlike this latter group, the Holostipae are almost always brownish, golden, or blackish pigmented, rarely with a distinct reddish tinge to the complement.

Evans (1935) has postulated, correctly so in the writer's opinion, that the Holostipae represent the least modified, hence most primitive of the subfamilies of the Lejeuneaceae. This conclusion is warranted not only on the basis of stem-structure but is also supported by the following points of evidence: (1) rarity of asexual reproduction, compared with the Schizostipae and Paradoxae; (2) frequent presence of secondary pigmentation; (3) robust size; (4) retention of large underleaves; (5) presence of a perianth that is fundamentally trigonous in most of the more primitive genera; (6) least reduction in the number of cell-rows in the seta of the sporophyte.

Only seven genera of Holostipae occur in the region here treated. Furthermore, four of these genera are monotypic locally, while two of the remaining three include only two local species each. As a consequence, the group offers no special taxonomic problems locally.

HOLOSTIPAE

KEY TO FERTILE PLANTS

1. Inflorescences on an elongate or short shoot, without subfloral innovation; cells with numerous small, homogeneous oil-bodies ..... 2.

2. Perianth 3-carinate, on an elongate shoot; perianth more or less compressed, the keels smooth or dentate; lobule with a single apical tooth; developing discoid gemmae of the antical surface of the lobes. No secondary pigmentation ..... *Caudalejeunea*
2. Perianth at maturity with 4–many carinae; with strong secondary pigmentation ....3.
3. Perianth strongly dorsiventrally flattened, 4-carinate, the carinae winged, sharp, dentate or laciniate; lobule with a single, ill-defined, apical tooth; without caducous leaves ..... *Lopholejeunea*
3. Perianth terete, developing 4–12 carinae extending the length of the perianth, the carinae obtuse, smooth; lobule with 2-several marginal teeth; with caducous leaves (in our species) ..... *Ptychocoleus*

1. Inflorescences becoming pseudolateral through the development of a strong subfloral innovation, or with 2 subfloral innovations (and then situated in the fork of a dichotomy) 4.
4. Perianth usually with 8–16 rounded plicae; free margin of lobule bearing a number of small denticulations; oil-bodies numerous, small, homogeneous: ♀ inflorescences subtended by two innovations, the perianth in the fork of a dichotomy *Brachiolejeunea*
4. Perianth with 2–5 carinae; lobule marked by a single apical tooth; oil-bodies large, 1–5 per cell, papillose to segmented; perianth subtended by a single innovation, becoming pseudolateral ..... 5.
5. Perianth with 2–3 carinae (0–1 ventral, 2 lateral); with strong secondary pigmentation, the plants fuscous; oil-bodies 2–5 per cell; lobule with hyaline papilla proximad of apical tooth or ental ..... 6.
6. Apical tooth of lobule acuminate, prominent; posterior leaf-margin sharply notched at juncture of keel and free dorsal lobe; perianths with lateral (and sometimes ventral) keels produced as distal auricles or horns .... *Neurolejeunea*
6. Apical tooth obscure, usually ill-defined; posterior leaf-margin evenly continuous from keel to free dorsal lobe; perianth with smooth keels, not produced *Mastigolejeunea*
5. Perianth 4–5 carinate (2 ventral, 2 lateral, usually with 1 dorsal distinct); without secondary pigmentation; oil-bodies coarsely segmented, large, 1 per cell; lobule with hyaline papilla distad of apical tooth, situated on inner margin of apical sinus *Leucolejeunea*

ARTIFICIAL KEY TO STERILE PLANTS

1. Lobule with 3–6 denticulations of the free margin; oil-bodies homogeneous, minute, many per cell; leaves more or less squarrose when moist ..... 2.
2. Plants blackish-green to black; leaves persistent. (Only in southern tip of Florida) *Brachiolejeunea*
2. Plants golden- to yellowish-green, pale brownish with age; leaves of erect, stiff shoots caducous. (Only in central Florida) ..... *Ptychocoleus*

1. Lobule at most with a single apical tooth distinct ..... 3.
3. Plants black or brownish, strongly pigmented ..... 4.
4. Lobule with apical tooth sharp, curved, prominent; underleaves distant, small (less than twice stem width); cells each with 2–4 very large, finely papillose-appearing oil-bodies ..... *Neurolejeunea*
4. Lobule with a small, inconspicuous apical tooth; underleaves very large, 2–3 times as wide as stem, often imbricate or approximate ..... 5.
5. Underleaves essentially obdeltoid, subtruncate distally; cells each with 2–4 large, coarsely papillose or segmented oil-bodies; leaves convolute and erect when dry, subsquarrose when moist; plants with secondary stems ascending, free from substrate; leaves dull ..... *Mastigolejeunea*
5. Underleaves essentially reniform, much broader than long, rounded distally;

each cell with numerous minute, homogeneous oil-bodies; leaves flat both when wet and when dry, neither convolute nor squarrose, shiny when dry; plants with secondary stems prostrate like primary..... *Lopholejeunea*

3. Plants green to gray-green or olive-green, never with distinct secondary pigmentation; lobule marked at apex with a distinct, often sharp tooth.....6.
6. Underleaves emarginate at apex; lobule with hyaline papilla proximad of apical tooth; cells with numerous minute, homogeneous oil-bodies. (Only in southern Florida)..... *Caudalejeunea*
6. Underleaves entire; lobule with hyaline papilla distal to apical tooth; cells each with a single very large, segmented oil-body. (Widespread)..... *Leucolejeunea*

KEY TO LIVING PLANTS

1. Oil-bodies 12–30 per cell, minute, homogeneous.....2.
2. Lobule with free margin bearing 3–6 small teeth; plants more or less distinctly pigmented; leaves squarrose when moist, convolute when dry.....3.
3. Pigmentation blackish-green; leaves never caducous..... *Brachiolejeunea*
3. Pigmentation golden or yellowish, with age pale brownish; with short erect, caducous-leaved shoots..... *Ptychocoleus*
2. Lobule with free margin entire, except for the sole apical tooth; leaves flat, both when dry and when moist.....4.
4. Plants shiny when dry, fuscous to blackish; underleaves broad and reniform-orbicular, rounded distally; plants uniformly prostrate..... *Lopholejeunea*
4. Plants dull when dry, green to olive-green; underleaves little broader than stem, emarginate at apex; secondary stems arching away from substrate *Caudalejeunea*

1. Oil-bodies 1–4 per cell, papillose to segmented, very large.....5.
5. Oil-bodies 2–4 per cell, more or less papillose, less than 6 x 10 μ; plants brownish to fuscous pigmented.....6.
6. Underleaves distant, less than twice stem-width; lobule at apex with a sharp, acuminate apical tooth; plants flat, whether dry or moist, closely adnate to substrate..... *Neurolejeunea*
6. Underleaves imbricate, at least twice stem-width; lobule with a slight apical tooth; plants with secondary stems ascending; the leaves erect and convolute when dry, squarrose when moist..... *Mastigolejeunea*
5. Oil-bodies 1 per cell (rarely with a small supplementary one), coarsely segmented, over 6 x 12 μ; plants without secondary pigments, green..... *Leucolejeunea*

SUBFAMILY SCHIZOSTIPAE (Spruce, 1885)

Subtribe Tomiae Frye and Clark, Univ. Wash. Publ. Biol. **6:** 789, 1947.

Minute to medium-sized, rarely robust plants (usually 200–1500 μ wide), usually *yellow-green to whitish-green*, rarely with golden to reddish-brown secondary pigmentation. Stems relatively slender, the cortical cells normally in 7 rows,[10] the medullary cells in 3-numerous rows; branching of the *Radula*-type only; *ventral merophytes almost without exception 2 cells broad*, normal in number (i.e. one ventral merophyte for each pair of lateral leaves. Leaves with lobes inserted by a long, very oblique incubous line, the insertion acroscopically arched ventrally and extending for some distance up the stem (the lobule, as well as the lobe, consequently broadly attached to the stem); lobes usually entire, occasionally acute

[10] In a few cases, such as in *Potamolejeunea orinocensis* St. the cortical cells are in up to 20 cell-rows. The systematic position of such taxa deserves further study.

or apiculate, more rarely with well-developed marginal teeth; lobules usually with a single apical tooth, the hyaline papilla at its proximal or distal base, not entally displaced. Cells not, to strongly, collenchymatous, the walls normally devoid of pigments; ocelli frequently developed (constantly so in many genera); oil-bodies very diverse, from 1–60 per cell, ranging from minute and homogeneous to enormous and compound. Underleaves small to large, *normally bilobed*, very rarely unlobed. Frequently with some form of asexual reproduction (caducous leaves; marginal, discoid brood bodies, etc.). *Perianths fundamentally 5-carinate*, never developing supplementary carinae, the antical keel often obsolete (the perianth becoming 4-carinate), and often with the two ventral keels united into a broad, biangulate, postical keel (then secondarily trigonous); bracteole large, usually bifid. Sporophyte with seta always formed of 12 epidermal rows surrounding 4 inner rows.

The Schizostipae include a large number of genera. Thirty genera are accepted in Vanden Berghen (1948); several have been described since, by Arnell (1953) (*Inflatolejeunea, Ciliolejeunea*) and Herzog (1948) (*Placolejeunea*). These genera, in very many cases, have exceedingly vague boundaries. Frequently the species of peripheral distribution (as regards the range of the genus as a whole) show the "generic characters" to a much slighter extent than the more strictly tropical species, which evolved apparently more recently, and represent the more highly derivative species of the genera. Excellent examples, among our species, are *Ceratolejeunea laete-fusca, Taxilejeunea obtusangula, Crossotolejeunea bermudiana* and *Harpalejeunea ovata*, all of which represent the most primitive elements in their respective genera. As a consequence, the student of the regional flora often gains only a limited idea of these genera. Associated with this phenomenon, we find that at the periphery of the distribution, in spite of a more limited representation of species, the determination of material is often very difficult, even to the correct genus.

In the region here treated, 13 genera are given for this group in the recent work of Frye and Clark (1947). One of these (*Caudalejeunea*) is incorrectly assigned by them. Of the remaining dozen genera, several (*Microlejeunea, Stylolejeunea, Euosmolejeunea*) are reduced to subgeneric standing in the following pages, leaving a total of nine genera for the area. The recent addition of *Hygrolejeunea* to our flora raises the total to ten.

ARTIFICIAL KEY TO GENERA AND SUBGENERA[11]

1. Dorsal lobes with ocelli present; lobule with hyaline papilla at proximal base of tooth ..... 2.

2. Underleaves widest near apex, the lobes divaricate, separated by a very shallow and

[11] This key, of necessity, depends largely on the characters of the sterile plant. This is due to the fact that of all of the taxa which key out under the first half of couplet 1 (*Drepanolejeunea* to *Microlejeunea* p. p.), perianths are known, in regional material, only for *Ceratolejeunea*, and in this species are produced only very rarely. Although the characters of the mature perianths offer excellent additional generic characters, their use would be totally impracticable.

broad sinus; leaves elliptical to narrowly ovate-lanceolate, more or less pointed distally, their length over 1.5 × the width; cell-walls colorless, not thick-walled (but often with distinct trigones and intermediate thickenings); pure green, delicate plants. 3.

3. Lobes of underleaves linear, formed of a single row of cells; body of underleaf (exclusive of lobes) bordered by 6 large cells; ocelli (in our species) not restricted to near the leaf-base; asexual reproduction via specialized caducous branches....4.
4. Dorsal lobes (in our species) acuminate, falcate; underleaves with the cells of the rhizoid-initial disk similar to the marginal cells; ocelli (in our species) 2–3 usually.....*Drepanolejeunea*
4. Dorsal lobes obtuse distally, elliptical; underleaves with marginal cells many times larger than the highly differentiated small cells of the rhizoid-initial disk; ocelli (in our species) often 3–8 per leaf.....*Leptolejeunea*

3. Lobes of underleaves rounded or obtuse, short, several cells broad; body of underleaf bordered by many cells; ocelli (in our species) in a sub-basal group; without specialized caducous branches.....*Harpalejeunea*

2. Underleaves suborbicular to ovate to reniform, widest near middle, the lobes separated by a narrow, acute sinus, erect or suberect; leaves ovate to suborbicular, their length less than 1.3 × their width; cell walls more or less equally thick-walled (except for thin-walled pits); with ocelli in a basal group.....5.
5. Underleaves large, their lobes 5–6 or more cells broad at base; cell-walls usually strongly thickened; lobules with apical tooth sharp, elongate, more or less falcate 6.
6. Leaves non-caducous (in our species), not developing marginal rhizoids; cell-walls brownish, with middle lamella usually deeper brown; dorsal lobes with margins entire to dentate; ocelli restricted to a basal group, or basal line. *Ceratolejeunea*
6. Leaves freely caducous, the caducous leaves on erect, rigid, short branches, the leaf-margins developing rhizoids; ocelli occurring in a basal group, and with scattered ocelli in the lamina as well.....*Rectolejeunea* subg. *Rectolejeunea*

5. Underleaves small, barely wider than stem, their lobes 2(3) cells broad at base; cell-walls usually slightly thickened; lobules with apical tooth short and blunt, straight or nearly so.....See *Lejeunea* subg. *Microlejeunea*

1. Leaves with cells uniform, quite lacking ocelli; leaf-lobes broadly ovate to subrotund, the margins normally lacking teeth, the apices narrowly to broadly rounded (rarely with a slight apiculum), the length of the lobes less than 1.4 × their width.....7.
7. Lobules (or at least a few of them) inflated and normal in form.....8.
8. Lobule with hyaline papilla at *distal* base of apical tooth; plants relatively xeromorphic, grey to olive-green, dull, their cells with large trigones (or equally thick-walled); cells with few (1–3, rarely 4–5) very large oil-bodies, thus quite opaque. *Cheilolejeunea*. 9.
9. Lobules (in our species) with a sharp, acute, 1-celled apical tooth 3–4 × as long as wide, usually somewhat falcate; underleaves always relatively small, usually distant, never cordate or auriculate at base; perianth strongly dorsiventrally compressed, the antical keel absent or vestigial.....Subg. *Cheilolejeunea*[12]
9. Lobules with a blunt, 1-celled apical tooth 1–1.5 times as long as wide, straight; underleaves small to large and imbricate, often somewhat cordate or auriculate at base; perianth usually 5-carinate, but antical keel often vestigial.
Subg. *Euosmolejeunea*[13]

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[12] The genotype, *C. aeneogyna*, belongs here, as well as the African *C. tisserantii* Jovet-Ast and Vanden Berghen. Of our species, only *C. decidua* is to be placed in *Eucheilolejeunea*.

[13] Here are assigned not only the species previously placed in *Euosmolejeunea*, but many species recently placed in *Cheilolejeunea*, s. str.

8. Lobule with hyaline papilla at *proximal* base of apical tooth; plants not or slightly xeromorphic, whitish-green to yellow-green or pure-green, relatively pellucid, often more or less shiny; cells usually (but not always) with more, usually 4–60, oil-bodies of small or minute size, appearing relatively transparent.....10.
10. Leaf-apices (at least on weaker shoots) commonly apiculate; leaf-margins occasionally to frequently with at least one obscure tooth of the antical margin, just below the apical tooth; cells with nodular trigones and free development of nodular intermediate thickenings; perianths specialized (either with dentate keels, or without a beak); our species autoecious.....11.
11. Underleaves large, cordate to auriculate at base, broader than long, much broader than stem; perianth with the 5 keels obscure (in our species) and without a beak (in our species).....*Taxilejeunea*
11. Underleaves small, little wider than stem, cuneately narrowed or rounded to base, longer than broad; perianth with the 5 keels faintly to distinctly denticulate or dentate, with a distinct beak.....*Crossotolejeunea*
10. Leaf-apices normally narrowly to broadly rounded, the margins normally quite entire; cells rarely with free development of intermediate thickenings; perianths simple: with 4–5 distinct, smooth or faintly crenulate keels and a distinct beak.....12.
12. Leaves, at least in small part, caducous (at least on some shoots); perianths obcordate to strongly obovate, widest distally, strongly flattened and with antical keel low or obsolete, thus essentially 4-carinate; plants dull, relatively opaque, usually with few (3–5 oil-bodies per cell).
Rectolejeunea subg. *Heterolejeunea*
12. Leaves uniformly persistent; perianths distinctly 5-carinate, little or not dorsiventrally compressed, the antical keel usually sharp; plants whitish to yellowish-green or pure green, relatively bright, usually relatively pellucid.....13.
13. Underleaves extremely large, imbricate, rounded to subcordate at base, bilobed by a very narrow, often closed, shallow sinus (the lobes usually contiguous, often connivent); lobes strongly deflexed, somewhat convolute, somewhat cordate at base. Plants large (1.0–2.0 mm. wide), but extremely delicate and flaccid.....*Hygrolejeunea*
13. Underleaves small to medium-sized, seldom imbricate, usually cuneiform or narrowly rounded basally, bilobed by an open, often broad and deep sinus (the lobes never connivent or contiguous); lobes flat to moderately convex, but never deflexed-convolute, narrowed to base. Plants minute to small (200–1400 μ wide), never flaccid.....14.
14. Stem with medulla formed of numerous rows of cells; plants moderate in size: usually 600–1400 μ wide, with obliquely to widely spreading leaves (50–80°); lobules small to moderate in size (inflated ones 0.2–0.5 × the length of lobe, less than one-fourth the lobe area, not exceeding or barely exceeding the underleaves in size); cells usually with relatively numerous (5–60) oil-bodies per cell. Our species all autoecious, almost constantly fertile.
Lejeunea subg. *Lejeunea*
14. Stem with medulla formed of only 3 cell rows; plants small or minute; usually 175–500 μ wide, with erect to erect-spreading leaves (usually 5–35°, rarely 45–55°); lobules often large in size (their length then 0.5–0.8 × the length of lobe, 0.5–0.8 × the area of lobe, much larger than the very small underleaves); cells usually with few (1–5, rarely to 8–10) oil-bodies per cell. Dioecious; almost constantly sterile.....*Lejeunea* subg. *Microlejeunea*

7. Lobules (or almost all of them) reduced to small basal folds which may bear a linear apical tooth), not inflated.....15.
15. Lobules without a distinct, linear apical tooth (the apical tooth at most 1–2 cells long).....16.
16. Caducous leaves freely produced; perianths strongly dorsiventrally flattened. See *Rectolejeunea* subg. *Heterolejeunea*
16. Caducous leaves absent; perianth 5-carinate, although the dorsal keel often lower and shorter.....See *Lejeunea* subg. *Lejeunea*
15. Lobules with a linear apical tooth, 5 or more cells long, lying parallel to stem; caducous leaves absent; perianths strongly dorsiventrally compressed. *Lejeunea* subg. *Stylolejeunea*

Artificial Key (Based on Living Sterile Plants)[14]

1. Leaves obtuse, apiculate, or rounded at the apices, occasionally with a few faint teeth indicated.....2.
2. Cell-walls without obvious brownish pigmentation; middle lamella not conspicuous 3.
3. With ocelli distinct.....4.
4. Leaves elliptical, more than twice as long as wide; underleaves with lobes divaricate, formed of 2-3 superimposed cells, filiform....*Leptolejeunea elliptica*
4. Leaves broadly ovate to suborbicular or obovate, less than 1.5 × as long as wide; underleaves with erect or suberect lobes 2–several cells broad at base.....5.
5. Ocelli in a basal group only, usually 1–3 per leaf; underleaves with lobes only 2 (3) cells broad at base; leaves persistent *Lejeunea* subg. *Microlejeunea* (*L. bullata, ulicina, cardoti*)
5. Ocelli in a basal group of 6 or more, with scattered, smaller ocelli throughout the lamina; underleaves with lobes over 6 cells wide at base; leaves caducous. *Rectolejeunea* (*Rectolejeunea*) *berteroana*
3. Without ocelli, the laminar cells uniform.....6.
6. Cells with oil-bodies obviously segmented, 1–15 per cell.....7.
7. Leaves with inflated lobules always developed, bearing a hyaline papilla distad of apical tooth; cells with 1–3, rarely 4 very large (to 6 x 20 μ), coarsely segmented oil-bodies, thus appearing opaque.....8.
8. Leaves freely caducous, developing marginal rhizoids before dehiscing; apical tooth elongate, sharp.....*Cheilolejeunea* (*Cheilolejeunea*) *decidua*
8. Leaves persistent, or tardily caducous (but without marginal rhizoids developing); apical tooth of lobule short, blunt. (*Cheilolejeunea* subg. *Euosmolejeunea*).....9.
9. Cells, or the majority of them, with a single, very large, coarse, oil-body. 10.
10. Cells with coarse, bulging trigones.....*C. clausa*
10. Cells without distinct trigones, more or less thick-walled. *C. parvula*
9. Cells with 2–3 (4) oil-bodies per leaf; trigones distinct. *C. rigidula, C. evansii, C. polyantha*
7. Leaves (or those with inflated lobules), with lobule bearing the hyaline papilla at proximal base of apical tooth; if lobule reduced, hyaline papilla at apex of apical tooth; cells with usually 4 or more smaller, segmented oil-bodies.....11.
11. Leaves (or some of them) caducous. *Rectolejeunea* subg. *Heterolejeunea*

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[14] In many instances sterile Lejeuneae have proved to be virtually impossible to name. The recent work by Schuster and Hattori (1954) on the oil-bodies has made possible the derivation of a key (in part direct to species), based on the vegetative plant (in part on inflorescences).

11. Leaves uniformly persistent.....12.
12. Lobules, or some of them, distinctly inflated.....13.
13. Medulla of stem with 3 rows of interior cells; shoots usually only 250–550 μ wide; underleaves with lobes 3–4 cells broad; leaves erect-spreading (*ca.* 30–50°); dioecious, usually sterile.
Lejeunea subg. *Microlejeunea* (*L. ruthii, laelevirens*).
13. Medulla of stem with numerous cell rows; shoots usually (on mature plants) 550–1400 μ wide; underleaves, at least of the largest shoots, with lobes 4–9 cells wide; leaves wide-spreading (50–85° usually); autoecious, usually fertile.....14.
14. Underleaves small to medium-sized, usually 1–2.5 as wide as stem, distant to contiguous (imbricate in *L. flava*), narrowly rounded or cuneate at base usually, bilobed 0.5–0.7 × their length by an open to U-shaped sinus, the lobes parallel or slightly divergent.....15.
15. Leaves uniformly rounded or obtuse at their apices, leaf-margins and underleaf-margins not to moderately crenulate; cells usually with small trigones and few intermediate thickenings; oil-bodies usually small (less than 5 x 10 μ) and fine-segmented.....*Lejeunea* subg. *Lejeunea*
15. Leaves (at least on some juvenile shoots or branches) apiculate or suddenly acute; leaf- and underleaf-margins strongly conspicuously crenulate; cells with large trigones and free development of intermediate thickenings, large and pellucid, very convex; oil-bodies large (to 6 x 15 μ), coarse-segmented.....*Crossotolejeunea bermudiana*
14. Underleaves very large (averaging two-thirds the area of the lobes), usually 2.5–3.5 × as wide as stem, contiguous to imbricate, broadly rounded to cordate at base, bilobed 0.3–0.45 × their length by an oval to linear, closed sinus, the lobes often distinctly connivent.....16.
16. Perianths without a beak, inflated and only obscurely 5-angulate near apex; cells with obvious trigones and intermediate thickenings; leaves at apices usually obtuse to acute, often with a faint indication of an antical, preapical tooth.....*Taxilejeunea obtusangula*
16. Perianths beaked, sharply 5-carinate in the distal two-thirds; cells with small trigones and rare or occasional, faint intermediate thickenings; leaves at apices uniformly rounded, without indication of any teeth.
Hygrolejeunea
12. Lobules reduced to small basal folds, the fold sometimes with an elongate apical tooth.[15].....17.
17. Lobule without a linear apical tooth formed of a row of 5 or more cells.
Lejeunea (*Eulejeunea*) *cladogyna, glaucescens*, and *floridana* may key here; some forms of *Rectolejeunea phyllobola* may key here.
17. Lobule with a linear, elongate apical tooth lying parallel to stem, 5 or more cells long.....*Lejeunea* subg. *Stylolejeunea*
6. Cells with numerous (usually 12–16, or up to 40–60) minute, glistening, homogeneous, elliptical to fusiform oil-bodies.....18.

[15] The fertile stems of a variety of Lejeuneae may have largely or entirely obsolete lobules. Mature sterile stems should therefore be studied in order to determine the size and form of normal lobules.

18. Leaves (or almost all of them) with distinct, inflated lobules; oil-bodies 30–60 per cell.....19.
19. Underleaves rounded or narrowed to base, as long as or longer than broad; cells usually with trigones and intermediate thickenings poorly developed.....*Lejeunea* (*Lejeunea*) *cavifolia*
19. Underleaves cordate to auriculate at base, broader than long; cells with distinct trigones and intermediate thickenings.....*Taxilejeunea*
18. Leaves (or almost all of them) with vestigial lobules; oil-bodies usually 8–30 per cell.....*Lejeunea* (*Lejeunea*) *minutiloba*

2. Cell-walls with obvious brownish pigmentation, the middle lamella usually a conspicuous, deeper brown; with a basal ocellus or group of ocelli.....*Ceratolejeunea*

1. Leaves narrowly ovate-acuminate, very sharp-pointed; ocelli present; underleaves small, widest distally, with divaricate lobes.....20.
20. Underleaf-lobes formed by 2–3 superimposed cells, uniseriate.....*Drepanolejeunea*
20. Underleaf-lobes several cells wide at base, rounded at apex.....*Harpalejeunea ovata*

SUBFAMILY PARADOXAE Lacouture (1908)[16]

Medium-sized to minute plants (250–1600 μ wide), green to yellowish-green in color (no secondary pigments), bearing leaves *with an exceedingly short line of insertion* which is almost transverse, rather than long and oblique as in other Lejeuneaceae. Stems delicate, the cortical cells in 5–7 rows; medullary cell rows reduced to either 3 or 1. Ventral merophytes of 2 or 1 cell-rows, twice the normal number, i.e. arising by the zigzag or pendulum-like, rather than spiral, mode of cutting-off of merophytes by the apical cell; *underleaves consequently twice the normal number* (but in groups with reduced ventral merophytes the underleaves quite absent); *rhizoid-initial fields* (often reduced to a group of 1–2 rhizoid-initials) *occurring one per lateral leaf*. Lobules inflated, with two teeth near apex. Cells not or little collenchymatous usually, sometimes nearly equally thick-walled, not developing secondary pigmentation; ocelli frequently developed (only in *Diplasiolejeunea* among our taxa); oil-bodies usually 3–12 per cell, small (largely less than 2–3 x 3–6 μ), coarsely segmented to almost homogeneous. Asexual reproduction only by discoid gemmae.

Androecia 1–2-androus. Perianths basically 5-keeled, but the antical often obsolete, the two postical sometimes weak and united to form a rounded postical keel (perianth then 3-carinate).

The Paradoxae include two distantly, but clearly allied groups of genera. The first group, to which belong *Colura* and *Diplasiolejeunea*, is characterized by the retention of large underleaves, which are typically deeply bifid, with divaricate narrow lobes. The second group includes *Cololejeunea*, *Aphanolejeunea*, *Leptocolea* (and such exotic genera as *Taeniolejeunea*); it is characterized by a less robust size, correlated with the absence of underleaves. This latter group, however, is undoubtedly most closely related to genera such as *Diplasiolejeunea* and *Colura* which are provided with one underleaf for every lateral leaf. This group, which has been called the Paradoxae, is maintained by most recent workers, including Evans (1935) and Berghen (1948, p. 48). Frye and Clark (1947)

[16] Excellent reasons for maintaining this group are given in Evans (1935).

place *Cololejeunea* (and the closely related *Leptocolea*) in the "Subtribe Aphylliae," on the basis of the absence of underleaves. The latter two genera are highly reduced groups, which, consequent upon their reduction in size, have lost the underleaves. Their derivation from a type like *Diplasiolejeunea* is clearly shown by the fact that a rhizoid-initial (or two adjacent rhizoid-initials) occurs on each of the ventral merophytes for each lateral leaf, as shown by Goebel (1928, p. 25). For that reason these genera are returned to the Paradoxae. The uniform pattern of asexual reproduction, and the constant occurrence, throughout the group, of a short, almost transverse, line of leaf-insertion are other characters that suggest a clear affinity.

The more primitive, less reduced, genera in the Paradoxae (*Diplasiolejeunea* and *Colura*) show a stem-anatomy quite distinct from that of the more reduced genera *Cololejeunea* and *Leptocolea*. In the less reduced, former, groups, the cortex consists of seven rows (*Diplasiolejeunea unidentata*, *Cololejeunea ornata*) or rarely six rows (*D. pellucida*) of cells (two of which belong to the ventral merophytes), and three rows of medullary cells. In the highly reduced *Leptocolea-Cololejeunea* complex, we find that the cortex consists of five rows of cells (only one row of which belongs to the ventral merophytes: the extreme to which we find reduction can proceed in types with a tetrahedral apical cell), and a single row of medullary cells. This separation into two groups, on the basis of stem-anatomy is fairly constant, but some species of *Leptocolea* appear to show an intermediate condition, with the cortex of six rows of cells (two belonging to the ventral merophytes), and the medulla of one row of cells; an example of this type is *Leptocolea planifolia* Evs. It is thus evident that the loss of underleaves is, consequent upon general reduction, correlated with reduction of the number of ventral cell-rows characteristic of the cortex, from two to one.

KEY TO GENERA OF PARADOXAE

1. Underleaves present, bifid, with narrow, divaricate lobes, one per lateral leaf; large plants, usually 1.2–1.6 mm wide; cells (in our species) dimorphic, some vegetative and with small oil-bodies, others forming ocelli and with a single large oil-body; stem with 2 rows of ventral cortical cells; leaves broadly rounded distally..... *Diplasiolejeunea*
1. Underleaves totally lacking; minute or small plants, 250–1200 μ wide; cells (in our species) uniform, lacking ocelli; stem with a single row of ventral cortical cells; leaves usually narrowly ovate or lanceolate, rarely broadly rounded distally.....2.
   2. Mature leaves with apical cells chlorophyllose, not modified; perianths not compressed, typically more or less 5-carinate; leaves (in most of our species) with the cells of the lobe, and sometimes of the lobule, more or less conically elevated or tuberculate (if cells not tuberculate, ventral lobe very large); plants more delicate, 250–800 μ wide.....3.
      3. Hyaline papilla *proximad* of apical tooth (i.e. in sinus between apical tooth and proximal tooth, usually at proximal base of apical tooth and slightly entally displaced; if lobules reduced, papilla at apex of apical tooth); apical and proximal lobular teeth distinctly separated by a broad sinus; leaves usually not dimorphic, vestigial lobules not regularly alternating with normal lobules; beak of perianth elongate, quite distinct. Gemmae usually on face of lobe, developing so that their faces lie parallel to the lobe-faces. Plants minute but not extraordinarily delicate. *Cololejeunea*

3. Hyalina papilla of lobulate leaves occurring *distad* of the apical tooth (situated at distal base of the 1–2-celled apical tooth, usually somewhat entally displaced); *apical and proximal lobular teeth closely approximated*, separated by a virtually closed sinus; *leaves markedly heterophyllous*, usually many elobulate and variously reduced; beak of perianth vestigial or absent. Gemmae restricted in development to marginal cells of lobes, developing with their faces at right angles to surface of lobe. Plants extraordinarily delicate; ocelli never developed.....*Aphanolejeunea*

2. Mature leaves with apical cells modified, forming a cluster of finger-like, hyaline cells (often absent on many leaves!); perianths dorsiventrally compressed, the lateral keels sharp and long, the antical keel low and reduced or obsolete, the postical keel 2-angulate and weak (often reduced to a single rounded keel); leaves (in our species) quite smooth, never tuberculate; plants relatively robust, 800–1200 μ wide when mature. (Structure of lobule as in Cololejeunea).....*Leptocolea*

NORTH AMERICAN LEJEUNEACEAE

II. PARADOXAE: THE GENERA APHANOLEJEUNEA AND LEPTOCOLEA[1]

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APHANOLEJEUNEA EVANS

Aphanolejeunea Evans, Bull. Torr. Bot. Club. **38:** 272, 1911
Cololejeunea (partim) *auctorum*

Plants excessively delicate, most often 300–800 μ wide, pure, usually pellucid green, often becoming whitish in drying. Stems prostrate, delicate, infrequently branched; *with five cortical cell-rows, surrounding a single medullary cell-row. Leaves polymorphic*, usually distant, rarely contiguous, the lobe distally often concave or canaliculate, usually spreading at an angle of 35–60 degrees, narrowly lanceolate to ovate to ellipsoidal, varying from acuminate to rounded at apex, the margin usually crenulate or denticulate from projecting cells; intramarginal cells often conically produced. Lobules usually *highly polymorphic*, some vestigial and 1–2-celled, others inflated; inflated lobules with the involute free margin often including the apex, sinus between apical tooth and keel lunate; apical tooth 1–2-celled, separated by a narrow and often slight sinus from the 1-celled proximal tooth (which is usually less distinct); *hyaline papilla situated distad of apical tooth* (usually at base of apical tooth, but slightly internally displaced, consequently not evident without dissection); keel arched, often denticulate in profile because of papillate cells. Cells usually exceedingly delicate and thin-walled (except for the sometimes thickened apices of the conical protrusions of some species), convex to conical; oil-bodies superficially homogeneous but fundamentally formed of delicate, minute, nonprotuberant, fine globules, minute (1.5–2 to 1.8–2 x 3–4.5 μ), oc-

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curring mostly 5–10, rarely 15 per median cell. Underleaves totally lacking. Asexual reproduction by discoid gemmae formed of 16–22 cells; gemmae originating *only from marginal cells of lobe*, the surface of the gemmae at right angles to surface of lobe.

Autoecious or paroecious (one species dioecious?). Female inflorescence on a main or short lateral branch, bearing one or two subfloral innovations; bracts unequally bilobed, complicate, the lobe similar to those of the leaves, the lobule various but plane. Perianth obovoid, terete or 5-keeled distally, rounded or angulate (but rarely horned) distally, abruptly contracted into a very short, frequently vestigial beak; surface of keels, or entire distal portion of perianth often roughened by projecting cells. Male bracts with lobules varying from vestigial (the antheridia then short-stalked and naked) to subequal to lobes in size (the antheridia then long-stalked and hidden).

TYPE: *Aphanolejeunea microscopica* (Taylor) Evans (= *Jungermannia microscopica* Tayl. in Mackay, Fl. Hibern. **2**: 59, 1836).

The genus has not been accepted by some recent workers, among them Macvicar (1926), Frye and Clark (1947) and Goebel (Ann. Jard. Bot Buitenzorg **39**: 34, 1928). Goebel stated that the position of the hyaline papilla in *Aphanolejeunea* (as well as in *Cololejeunea* and *Leptocolea*) is originally terminal on the lobule, and remains so in imperfectly developed leaves. As Evans (1938, p. 72) emphasizes, "In most cases, however, one or more cells adjacent to the papilla grow out and form the apical tooth, thus displacing the papilla to one side. . . . According to the writer's interpretation, however, the papilla is displaced to the proximal side of the apical tooth in *Cololejeunea* and *Leptocolea* and to the distal side in *Aphanolejeunea*." Evans also laid emphasis, as a generic character of *Aphanolejeunea*, on the fragility of the plant and the marked heterophylly of the leaves. In the opinion of the writer, the heterophylly of the leaves scarcely allows a separation of *Aphanolejeunea* from such species as *Cololejeunea diaphana* (which are, furthermore, scarcely less delicate). The position of the hyaline papilla, although constant, is by itself scarcely sufficient to warrant retention of the genus as distinct from *Cololejeunea*. Consequently, the claims of *Aphanolejeunea* for generic recognition leave much to be desired; future reduction to subgeneric standing under *Cololejeunea* may prove desirable.

The genus *Aphanolejeunea* includes the most delicate and most easily overlooked of our Lejeuneaceae. The closely prostrate growth and exceeding delicacy result in the plants' being overlooked in the field as well as contribute greatly to the difficulty in studying the material. Furthermore, the plants almost invariably occur sparsely as scattered stems in admixture with other species, with the consequence that adequate material for dissection is rarely available. The minute size and diffuse and sporadic occurrence have resulted in the description of species from only one or two collections, with a consequent too narrow definition of the species. Careful study has shown considerable latitude in modification within at least one species. Species concepts in the genus must therefore be considered as fluid and subject to considerable future modification. Up to the present, the genus has been known from the United States solely on the basis of two reports of *A. sicaefolia*, from Dade and Seminole Counties, Florida.

While collecting tropical Hepaticae in southern Florida, the writer has found

several small colonies of an extremely minute member of the genus at first identified doubtfully as *Aphanolejeunea sicaefolia* (Gottsche) Evans.

Repeated careful study of the material, however, revealed certain constant differences that suggest that the plants belong to two discrete species, described below. Furthermore, neither of these species is conspecific with *A. sicaefolia*. Although the latter has been reported from Florida (Evans, 1915; Frye and Clark, 1947), all the material previously reported that has been available for study has proved different from *A. sicaefolia* (as typified in Evans, 1911). The relationships of the two species here reported from Florida to *A. sicaefolia*, and to the other West Indian and Oceanic species, are summarized in the following Key.

KEY TO THE SPECIES OF APHANOLEJEUNEA IN NORTH AMERICA, WEST INDIES AND EUROPE[2]

1. Mature leaves (with inflated lobules) with apical teeth of lobules formed of 2 cells, the hyaline papilla at the base of the lower of these 2 cells (Fig. I: 5); mature, lobulate leaves short, ovate-lanceolate to broadly lingulate, *ca.* 1.5–2.4 as long as wide, the apex acute to obtuse, rarely narrowly rounded, never acuminate; lobules of mature leaves large, their length 0.5–0.85 × the length of the dorsal lobe (Fig. I: 3, 8).....2.
   2. Lobule 0.6–0.8 × the length (and area) of the dorsal lobe; perianth without keels, smooth or with conical or papillose protuberances on distal half; leaves narrowly ovate to broadly lanceolate, widest below middle, margins not parallel.....3.
      3. Cells strongly convex, and at least the marginal more or less conically protuberant (the leaf-margins thus strongly crenulate or serrulate); keels strongly crenulate from the conically elevated cells; perianths distinctly papillate above (Florida and West Indies).....4.
         4. Cells of antical leaf-margin, especially at and above middle of lobe, large (20 μ high), larger than the interior cells; leaves very strongly crenulate-dentate along both antical and postical margins, on keel, and on antical surfaces from sharply conical cells. (Imperfectly known; sterile).
            A. verrucosa Jovet-Ast[3] (Guadaloupe)

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[2] *A. sintenisii* (St.) comb. n. (= *Lejeunea sintenisii* Stephani, Hedwigia **27**: 290–291, 1888) is described as dioecious and much branched. It is similar to *A. exigua*, but the lobe is larger (compared to the lobule), about twice as long as the lobule, and the projecting portion is larger (*ca.* 8 cells long × 6 cells wide; in *A. exigua* the lobe projects 3–5 cells beyond the lobule). Known only from sterile plants, its exact intrageneric disposition is impossible at present.

A. heterophylla (Goebel) comb. n. (= *Lejeunea heterophylla* Goebel, Pflanzenbiol. Schild. **1**: 178–179, fig. 79, 1889), from Puerto Rico, is equally impossible to place. Although not formally published, the descriptive notes and illustrations given by Goebel satisfy the then-existing rules as to valid publication of a new species. Evans (1911) considers this species to be related to *A. exigua* Evs., from which it is said to differ in the 5-keeled perianth. It is possible that the two are not specifically different.

The contributions most pertinent to our understanding of the American species are the classical work of Evans (1911) and the recent one of Mme. Jovet-Ast (1947).

[3] The characters separating this species from *A. exigua* Evs. are few and vague. Jovet-Ast stresses the more strongly "verrucose" dorsal face of the lobe, and the "plus profondément crénelé" margin of the dorsal lobe. My experience with hundreds of specimens of *Cololejeunea biddlecomiae* has shown that the degree of development of the conical papillae of the leaf-cells is directly related to environmental conditions. The range in this last species suggests that it may be necessary to unite *A. verrucosa* and *A. exigua*.

4. Cells of leaf-margins no larger than interior cells, 10–15 μ wide; leaves slightly to moderately crenulate on margins, the conical protuberances usually only locally developed (if present throughout, only moderately elevated).....5.
5. Autoecious or dioecious. Leaves often lacking lobules, often reduced to narrow blades 2–4 cells wide; perianth without a beak; male bracts concave, in 3–5 pairs; female inflorescence with one subfloral innovation; perianth at maturity not or barely extending beyond bracts.....6.
6. Male bracts well-developed, the lobules large, formed of numerous cells (Fig. I: 6, 7); ♀ bracts shorter than perianth, with subacute to rounded apices, the lobes 6–8 cells broad, the lobules 4–5 cells broad and 0.7–0.9 × as long as lobes (Fig. II: 5–7); elobulate leaves elliptical to ovate to short-rectangular (Fig. II: 2, 9); leaves with marginal as well as non-marginal cells usually conical (Fig. II: 2–3). Autoecious.
A. ephemeroides sp. n. (Florida)
6. Male bracts small, the lobules reduced to 5–10 cells, up to 0.5 × length of lobes; ♀ bracts as long, or nearly so, as perianth, linear to linear-lingulate, narrowly tapering and acute at apex, only 4–5 cells broad, the lobules 2–3 cells broad, and 0.4–0.5 × as long as lobes; elobulate leaves often linear (2 cells wide x 6–8 cells long); leaves with marginal cells and cells of keel weakly conical, the surface cells of lobe not produced. Apparently dioecious.....*A. gracilis* Jovet-Ast. (Guadaloupe)
5. Paroecious. Plants with leaves usually lobulate; perianth beaked; male bracts a single pair, below ♀ inflorescence; ♀ inflorescence with 2 subfloral innovations; perianth at maturity extending far beyond bracts.
A. exigua Evs. (Puerto Rico)
3. Cells slightly convex, never conically produced, the lobe margins entire or vaguely crenulate; keels with cells at most evenly convex, never conically elevated. (Europe, Azores).....7.
7. Perianth provided above middle with discrete conical papillae; lobule large, *ca.* 0.8 × the dorsal lobe in size; apical tooth of lobule short (18–20 μ long), little or not curved; "gemmae" *ca.* 90 x 70 μ, 16-celled, developed "on the surface of the leaves;" paroecious.....*A. microscopica* (Tayl.) Evs. (Europe, Azores)
7. Perianth smooth; lobule varying from slightly more to slightly less than 0.5 × the area of the dorsal lobe; apical tooth of lobule elongate, 25–35 μ long, curved; "gemmae" 130 × 100 μ, of 19–22, usually 20 cells, arising from marginal cells only; autoecious.....*A. teotonii* V. Allorge et S. Jovet-Ast. (Azores)
2. Lobule *ca.* 0.5 × the length (and area) of the dorsal lobe on mature leaves; perianths obscurely 5-keeled near apex, the keels slightly roughened by projecting cells, the surface between keels smooth; leaves lingulate to lingulate-oblong, their margins subparallel for some distance, faintly crenulate by projecting cells, but never with conically elevated cells either on margins or on surface; one subfloral innovation. Paroecious.....*A. crenata* Evs. (Puerto Rico)
1. Lobulate leaves with apical teeth short, formed of a single cell, the hyaline papilla at the base of the solitary apical cell (Fig. I: 1); mature, lobulate leaves usually elongate, ovate-lanceolate to narrowly lanceolate, (2.1) 2.5–4 × as long as wide, the apex acute to acuminate; lobules of mature leaves relatively small, their length 0.25–0.45 (rarely 0.5–0.6 × the length of dorsal lobe (Fig. I: 1, III: 2, 6). Autoecious.....8.
8. Lobes usually terminated by 1 (rarely 2) cells (Fig. II: 6); ♂ bracts leaf-like, with lobule well developed, inflated, 0.25–0.6 × the length of the lanceolate lobes (Fig. IV: 1, 5); perianths smooth (except, sometimes on the keels, if the latter present).....9.
9. Lobes narrowly lanceolate, 3–4 × as long as lobules, totally devoid of conical protuberances; perianths smooth, shortly to longly obovoid, devoid of keels; median cells of lobe 18–30 x 40–60 μ..... *A. longifolia* Jovet-Ast
9. Lobes lanceolate to ovate-lanceolate, 1.5–2.5 × as long as lobules, some marginal cells of lobes (and often of and near keels) with sharp conical protuberances (Fig.

III: 2, 6); perianths sharply 5-carinate, the keels produced as angulate shoulders or horns, their margins denticulate (Fig. III: 2-3); median cells of lobe 12-16 x 22-35 μ..... *A. evansii* sp. n. (Florida)

8. Lobes usually terminated by 2-4 superimposed solitary cells, narrowly acuminate (Fig. I: 1); ♂ bracts reduced, their lobules of 1-2 cells, the single antheridium naked; perianths with weak, rounded keels, both keels and surface between them strongly papillate; leaves 3-4 times as long as lobules, the marginal cells of keel and sometimes interior cells of lobe conically elevated.
A. sicaefolia (Gottsche) Evs. (Cuba, Puerto Rico, Jamaica, Guadaloupe to Trinidad)

Aphanolejeunea ephemeroides Schuster sp. n.

Viridis, gracilis (300–350 μ lata) folia heterophylla, ea sine lobulis parva, longa 6–8 cellis, lata 4–5 cellis; folia lobulata grandiora, lobis ad 140–180 (225) μ longis x 75–85 μ latis, parum ovata, dein ovato-lanceolata, acuta, subacuta vel parum apice curvata; lobuli magni 130–135 μ longi x 75 μ lati, 0.6–0.85 longitudinis lobulorum dente apicis falcato, 2 cellularum elongatarum. Cellulae leptodermatae, 10–15 x (14–17) 15–20 μ ad medium; cellulae marginales, cellulae carinae et saepe cellulae in parte distale lobuli productae in conis. Autoiciosa; cum una (1) subflorali innovatione; bractea polymorphia, lobi anguste ovati ad oblongatos, subacuti ad obtusos vel rotundatos; lobuli minime breviores lobo, acuti vel subacuti. Perianthia obovoida, sine carinis vel rostro, partem distalem superne subundulatam porrectis tuberculis.

Shoots strongly heterophyllous, up to 300–350 μ wide, sparsely branched, distantly leaved. Stems 25–30 μ in diam., distinctly zig-zag angulate, filiform. Leaves of two types: elobulate small, 6–8 cells long x 4–5 cells wide, and lobulate larger, suberect, their lobes up to 140–180 (225) μ long x 75–85 μ wide; lobulate leaves with lobules large, 130–135 μ long x 75 μ wide, inflated, 0.6–0.85 × the length and area of the lobes, terminated by a hooked apical tooth formed of two cells (the outer of which is 8–9 x 20–24 μ), much exceeding the inconspicuous 1-celled proximal tooth; distal, falcate tooth of lobule closely applied to dorsal lobe, forming with the surface of the lobe and sinus of the lobule a discrete, nearly circular opening to the water-sac formed by the lobule; lobes narrowly ovate to broadly lanceolate, acute to subacute to obtuse, rarely rounded distally, *terminated by a single solitary cell*. Cells pellucid, extremely thin-walled, devoid of trigones, 10–15 x (14–17) 15–20 μ medially; marginal cells, cells of keelar regions, and often cells of distal one-half of lobes (and of ♀ bracts) more or less conically produced, the leaves thus distinctly roughened. Oil-bodies virtually homogeneous in appearance (under oil-immersion evidently formed of exceedingly fine, barely perceptible globules which do not protrude through the peripheral membrane), smooth, 4–9 per marginal cell, (2–4) 6–12 per median cell, spherical and 2–2.5 μ to, more often, ovoid to fusiform and 1.5 x 2–2.5 to 1.2–1.5 x 4–5 or 2 x 4 μ. Chloroplasts subequal to oil-bodies in size, *ca.* 3–3.5 μ long. Asexual reproduction by discoid, 16-celled gemmae abundant; gemmae small, *ca.* 42 x 52 μ, a flat plate formed only from marginal or apical cells of lobe, lying at right angles to lobe-surface.

Autoecious. Androecia variable, formed of 3–6 or more pairs of bracts, spicate, much narrower than vegetative shoots, *ca.* 170–175 μ wide, terminal on usually a short, lateral branch (never or rarely resuming vegetative growth after maturation of bracts); bracts saccate, contiguous to barely imbricate, erect or suberect, usually nearly equally bilobed; dorsal lobe 80–95 μ long x 65 μ wide, obtuse or rounded at apex (or slightly retuse, with hyaline papilla in emargination); antheridia single, 38–40 μ in diam. Female inflorescences on main shoots, typically with a single subfloral innovation; ♀ bracts polymorphic, the lobes from a

minimum of 180–200 μ long x 70–80 μ wide, with lobule 140–150 μ long x *ca.* 50 μ wide, to lobes 245–260 μ long x 100 μ wide, with lobule 190–200 μ long x 50 μ wide; lobes lingulate or narrowly rectangulate to narrowly elliptical, little longer than the narrow, fold-like lobules; lobe-apices usually subacute to obtuse

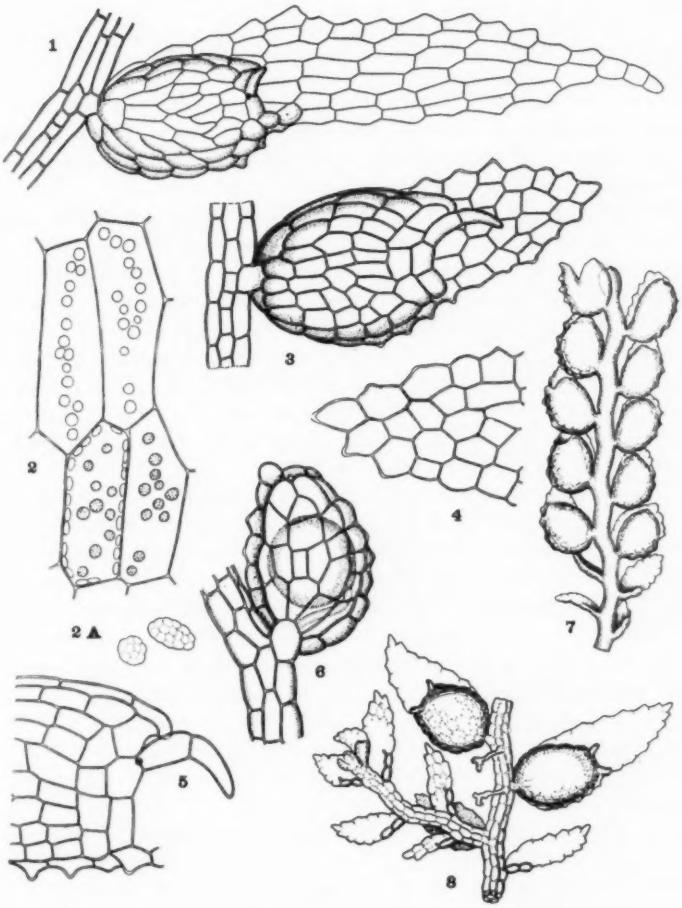

FIG. I. *Aphanolejeunea sicaefolia* (Gottsche) Evans (El Yunque, Puerto Rico). 1. Leaf, *in situ* (× 200); 2. Median cells with oil-bodies; chloroplasts in lower left-hand cell (× 1130); 2a. Individual oil-bodies (× 2300). *Aphanolejeunea ephemeroides* Schuster 3. Leaf, *in situ* (× 300); 4. Apex of leaf-lobe (× 375); 5. Apex of lobule, the hyaline papilla stippled (× 300); 6. Male bract, postical view (× 300); 7. Androecial branch, postical view (× 90); 8. Shoot-sector, with branch, postical view (× 150). (Figs. 1–2, El Yunque, Puerto Rico; 3–8, Paradise Key, Florida, *Schuster 22590a*.)

to rounded, distinctly denticulate owing to projecting cells; surfaces of lobes, distally more or less armed with projecting tubercles; ♀ bracts typically shorter than the mature perianth, usually ⅔–¾ × as long. Perianth obovoid, at maturity often distinctly stipitate, rounded distally and ecarinate as well as erostellate, the distal one-fourth to one-half roughened owing to projecting, tubercular, cells.

TYPE: Over base of trunk of *Quercus laurifolia*, ravine of Gold Head Branch State Park, Clay Co., Florida, *R.M. & O.M. Schuster 31405*, Dec. 31; 1953. COTYPE: same data, *RMS 31407*. ISOTYPE: Paradise Key, Everglade Keys, Dade Co., Florida, *Schuster 22590a*.

Aphanolejeunea ephemeroides, at first glance, gives the impression of being an impoverished phase of either *A. sicaefolia* or *A. evansii*. It has (on lobulate leaves) the surface-area ratio between lobe and lobule reduced (*ca.* 1.2–2.0:1), and has merely acute, subacute or even obtuse to rounded dorsal lobes. On that basis, the plants of this species were confused, at first, with poorly developed plants of *A. evansii*. With this species they agree in a number of characters, among them: (1) male bracts with large inflated lobules; (2) leaf-lobes with apex formed by a solitary cell; (3) small size, with the leaves rarely over 200 μ long in *A. ephemeroides*. However, careful comparison shows the following major points of difference: (1) perianths as in *A. sicaefolia* and *gracilis*, i.e. virtually non-carinate, all or most cells of the distal portions of the perianth conically produced; (2) male bracts suberect, subequally bilobed, the lobe short and obtuse or subacute at apex; (3) lobules of normal leaves with apical tooth hooked and formed of 2 cells, rather than unicellular.

In all three of these features, the species approaches the recently described *A. gracilis* Jovet-Ast, known only from Guadaloupe, in the French Antilles. It differs from *A. gracilis* as follows: (1) Male bracts large and well developed, with the lobules formed of numerous cells (compare Fig. II: 7–8 in Jovet-Ast, 1947, with Fig. I: 6–7 given here); (2) lobes of ♀ bracts with subacute to obtuse to rounded apices and with narrow, fold-like lobules, 0.7–0.9 × the length of the lobes (Fig. II: 5–7); (3) the elobulate leaves elliptical to ovate, commonly 4 cells wide x 5–6 cells long, to a maximum of 5 x 6–8 cells long; (4) ♀ bracts much shorter, never as long as the mature perianth and usually very distinctly shorter; (5) a strong tendency for the non-marginal cells of the lobes to become tuberculate, at least on the distal halves of the leaves and ♀ bracts. Jovet-Ast has emphasized, as one of the distinctive features of *A. gracilis*, the high incidence of sublinear elobulate leaves, which are very narrow (only 2 cells wide x 5–6 cells long near shoot-bases; up to 3–4 cells wide elsewhere, and there narrowly lingulate). In the material of *A. ephemeroides* no trace of such narrow and sublinear elobulate leaves has been found. The frequent (if not constant) occurrence of conical tubercles of the external faces of the lobular cells also appears distinctive; these appear to be absent in *A. gracilis*. In spite of these differences, study of a long series may show that *A. ephemeroides* should be regarded as conspecific with, or merely as a variety of, *A. gracilis*.

Also apparently closely allied is *A. verrucosa* Jovet-Ast (1947, p. 25, Fig. IV). Unfortunately, this species is known only from a few sterile fragments, which

differ from well-developed plants of *A. ephemeroides* as follows: (1) leaves nearly oval, widest near middle, suberect rather than spreading; (2) all cells of lobe strongly tuberculate, the marginal considerably larger (ca. 20 μ high) than the median; (3) lobular apical tooth 2-celled, but the cells not strongly elongate, the tooth not strongly falcate. As is readily evident, without fertile plants of *A. verrucosa*, no clear distinction between it and *A. ephemeroides* can be made. It may prove possible that both of these taxa, as well as *A. gracilis*, shall prove extremes of a single polymorphic species. The extent to which the conical cellular

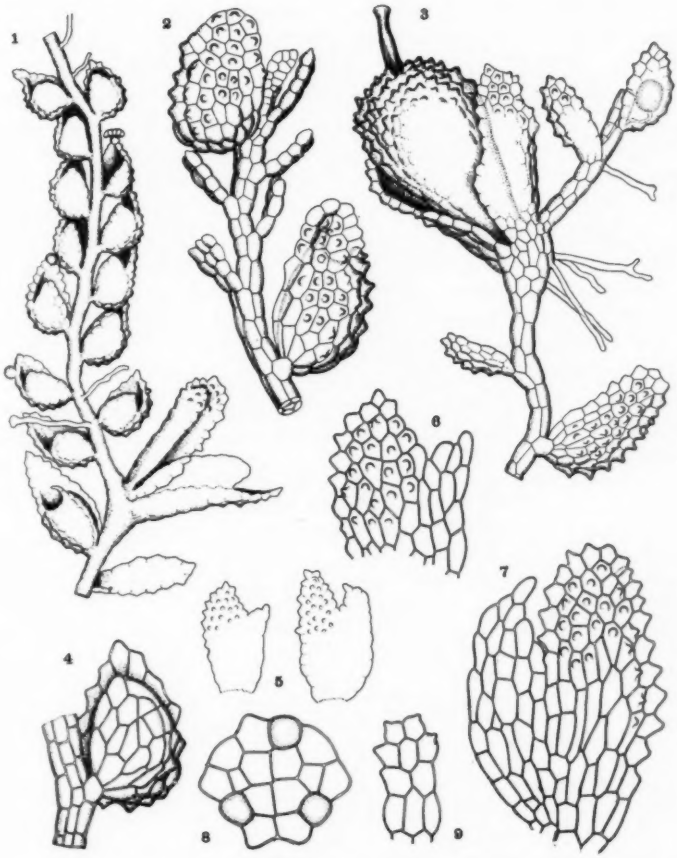

FIG. II. *Aphanolejeunea ephemeroides* Schuster. 1. Portion of plant, with androecium and gynoecium, postical view (× 80); 2. Apex of xeromorphic shoot, antical view (× 200); 3. Perianth-bearing shoot, with base of male innovation (× 200); 4. Perigonal bract (× 200); 5. Two female bracts (× 95); 6. Apex of female bract (× 200); 7. Female bract (× 200); 8. Gemma (× 440); 9. Elobulate leaf (× 290). (Figs. 1, 3–9, Clay Co., Florida, *Schuster 31405, type;* 2, 9, same locality, xeric extreme, *Schuster 31407*).

tubercles are developed is clearly subject to much environmental variation, and consequently is possibly over-emphasized in the literature. The more xeric extreme of *A. ephemeroides* (*Schuster 31407*; Fig. II: 2, 3) closely approaches that of *A. verrucosa* in that the leaves are suberect (when dry also standing somewhat stiffly away from the substrate and the stems), and have most lobar cells quite distinctly tuberculate (Fig. II: 2, 3). However, such xeric extremes of *A. ephemeroides* have smaller leaves (lobes *ca.* 120 μ long x 65 μ wide; lobule *ca.* 100 μ long x 55–60 μ wide), and the antical row of marginal cells are not strongly enlarged (compared with the adjacent interior cells) nor extremely conically elevated—conditions which characterize *A. verrucosa*. The writer has been quite unable to find plants matching those illustrated by Jovet-Ast (1947) under *A. verrucosa*.

A. ephemeroides is described as a species distinct from *A. verrucosa* only because the range of variation exhibited by the available material is so broad that it is very doubtful that this variability can encompass a type as extreme as that represented by *A. verrucosa*. The plants of *Schuster 31407* (Fig. II: 2, 3, 6, 7) are considered as the xeric phase, with strongly conically elevated cells and very short leaves, usually suberect in position, often rounded or obtuse at apex; these plants approach (but do not quite attain) *A. verrucosa* in the degree to which the cells are produced. The cotype material represents what is regarded as "typical" material, with the more divergent leaves acute or subacute, the cells of the keel and those of the margins of the lobe conically produced (but those of the lobe surface merely convex in most cases). The plants from Paradise Key (*RMS 22590a*) (Fig. I: 6–8) represent the mesic extreme, with the leaves acute, weakly crenulate or denticulate on the distal portions of the margins, and the keels weakly denticulate in profile.

Aphanolejeunea evansii Schuster sp. n.[4]

Viridis, gracilis (250–525 μ lata); folia polymorphia, lanceolata ad lanceolato-acuminata, ad 275 μ longa x 110 μ lata; lobuli saepe obsoleti quando inflati, cum apice signato dente apicali unae cellulae; cellulae leptodermatae 14–15 (16) x 25–30 μ (35) μ in medio folii; cellulae marginales loborum et cellulae in et ad carinas elevatae in conem. Autoiciosa, una (dua) innovatione subflorali, bractea lobis acuminato-lanceolatis, lobulus 0.5–0.65 longitudinis lobi. Perianthia obovoida, 5-carinata, carinae superae in angulo productae, margines denticulatae, cellulis acute productaes; aliter perianthia superne lena, rostrum breva sed distinctum. Foliae ♂ completae, paene parieter bilobae, non facientes elongatum spicatum androecium.

Pure transparent green; growing in a loose, delicate arachnoid film, or scattered among other Hepaticae. Stems moderately zigzag, 25–35 μ in diam., loosely and irregularly pinnate, the branches similar to the main stem, diverging at an angle of 75–95°; plants with leaves varying from 250 to a maximum of 525 μ in width (on fertile shoots); ventral cortical cells 17–18 x 28–35 μ. Rhizoids sparsely developed, at most one per leaf on robust shoots. Leaves polymorphic, lanceolate to lanceolate-acuminate, often slightly falcate on robust shoots, the apex more

[4] After Dr. A. W. Evans, in admiration of his painstaking and exact work on the members of this family, which stands as a model for all future work.

or less contorted or decurved; leaves 175–275 μ long, 75–110 μ wide; dorsal lobe on well-developed lobulate leaves with the antical and postical free margins both somewhat dorsally reflexed, the lobe thus somewhat longitudinally sulcate; margins of the lobe, beyond lobule, more or less crenulate-serrulate, from angularly protuberant cells, some of which are (at least on well-developed leaves) slightly produced as a conical outgrowth; cells of lobe interior to the margin convex, but apparently never or at best very rarely similarly conical; apex of lobe almost invariably terminating in a single cell, never in a row of 2–3 cells. Lobule elliptical to ovate, *ca.* 85–125 μ long x 70–90 μ wide, its cells convex, except for those of the outer portions of the keel, which may be weakly to strongly conically produced in well-developed leaves; lobule distally terminated by an elongate cell with decurved apex, marking the proximal tooth, separated by a slight indentation from an equally small distal tooth formed of a single cell. Elobulate leaves lanceolate to ovate-lanceolate, from 4 cells and 50 μ wide x 120 μ long to 6–7 cells wide, and 100-115 μ wide x 195–220 μ long; lobules reduced to a 2-celled ridge, at whose apex occurs the hyaline papilla. Cells of free half of dorsal lobe elongate, averaging (12) 14–15 (16) x (22) 25–30 (35) μ, extremely leptodermous, without trigones or intermediate thickenings, colorless; marginal cells irregularly quadrate-polygonal, (12) 16–18 x 18–20 μ, near the leaf-apex becoming 13–17 μ. Median elongate cells of free lobe with 7–12 oil-bodies per cell, marginal and submarginal cells with 4–6 (rarely to 10) oil-bodies per cell; oil-bodies minute, mostly spherical or short-ovoid, varying from 1.6–2 μ and spherical to 1.8 x 3 μ (and short-ovoid to broad-ellipsoidal), a very few to 2 x 4.5 μ, appearing nearly smooth and homogeneous, glistening (but under oil-immersion barely perceptibly formed of a small number of scarcely apparent globules that do not protrude through the bounding membrane); the oil-bodies thus virtually smooth in appearance. Chloroplasts averaging distinctly larger in surface area than oil-bodies. Asexual reproduction by means of marginal, many-celled, discoid "gemmae" produced on the margins of the dorsal lobes on mature leaves, rarely on immature or reduced ones, first evident when a marginal cell becomes spherical and turgid and its cell-contents become more opaque than that of surrounding vegetative cells; subsequently, a vertical cell-division parallel with the leaf-margin occurs, followed by a vertical division of at least the outer of the two daughter cells (at right-angles to the preceding division); subsequent divisions result in a circular, discoid "gemma" consisting, at time of abscission, of 16 cells, with 3 rounded slime-cells, the other marginal cells often angularly produced; gemmae *ca.* 50 x 56–64 μ.

Autoecious. Female inflorescence usually on an elongate branch or main shoot (Fig. I: 2) more rarely on a short, lateral branch, innovating at all times on one side, and sometimes (later) on the other side as well (Fig. III: 2), the innovations usually sterile, sometimes male; bracts suberect, with the distal portions slightly spreading, not strongly complicate, the dorsal lobe similar to that of leaves, but usually considerably longer ([220] 325–460 μ long x [60] 75–100 μ wide), the lobule ½–⅔ × as long as the lobe, 50–75 x (160) 225–250 μ, narrowly lingulate, rounded or shallowly truncate-bidentate at the apex; apex of dorsal lobe acute to briefly acuminate, terminated by a single cell (rarely a row of two superimposed cells); margins of lobe and lobule, beyond basal portions, crenulate-serrulate like leaf-margins. Perianth obovoid, *ca.* 300–420 μ long x 180–240 μ wide (in widest part at or near apex), distinctly 5-carinate on distal ½ to ⅔, the keels sharply angulate above, produced as shoulders or spreading, angulate horns; the margins of the keels sharply denticulate owing to high conical cells (which are *ca.* 14–16 μ wide along the margins), lying external to one or several cell-rows of strongly elongate cells that are not at all conical (Fig. III: 3); surface between keels quite smooth; perianth abruptly contracted

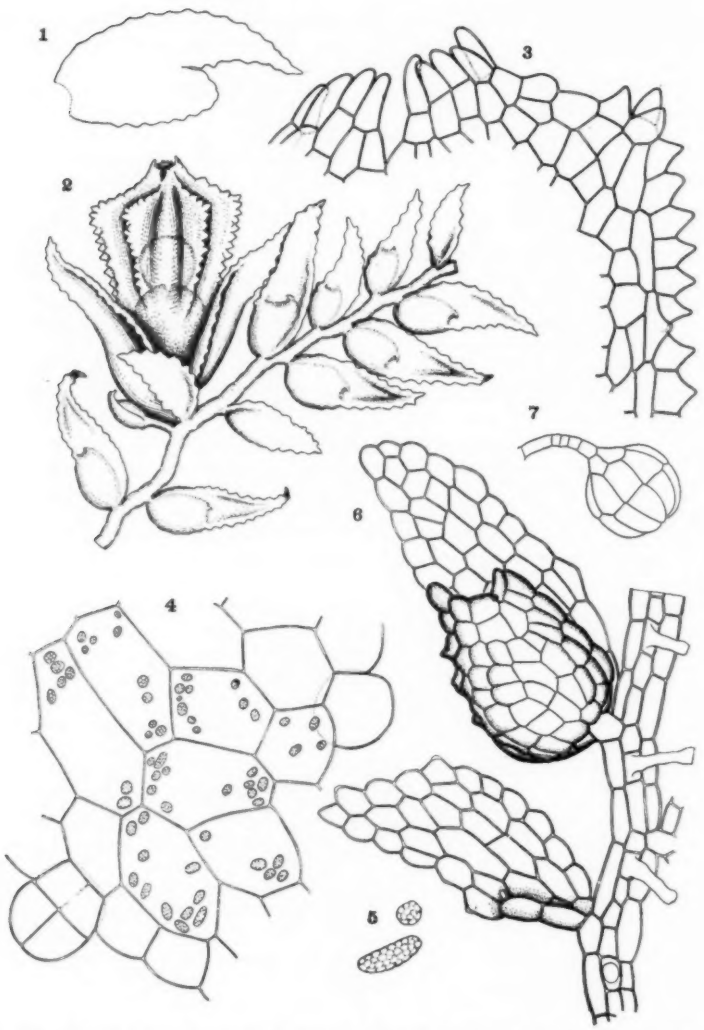

FIG. III. *Aphanolejeunea evansii* Schuster. 1. Female bract (× 140); 2. Perianth-bearing shoot, antical view (× 72); 3. Keel and beak of perianth (× 390); 4. Upper part of free lobe of leaf, showing oil-bodies in some cells, with two gemmae-initials (× 830); 5. Two oil-bodies (× 2200); 6. Sector of shoot, postical view (× 270); 7. Antheridium (× 350). (Figures all from paratype, Long Key Pineland, Everglades National Park, Florida, *Schuster 22029a*).

at mouth into a short beak that is crenulate with the free, rounded ends of cells that are 9–12 μ wide x 18–25 μ long, and coalesced laterally (for over ½ their length); perianth (before maturity of sporophyte) clearly longer than the bracts and projecting beyond them. Male inflorescences *ca.* 300–350 μ wide, on elongate

main shoots or subfloral innovations, of distant to loosely approximate, leaf-like bracts, bracts identical with leaves in form (lobe narrowly lanceolate, *ca.* 190 μ long x 70–75 μ wide; lobule *ca.* 85 μ long), the lobule inflated and bearing concealed a single antheridium; apex of lobule rounded, the hyaline papilla in a shallow sinus between the two apical cells. Seta 60 μ in diam., of 4 rows of interior cells, surrounded by 12 rows of epidermal cells of equal diameter.

TYPE:—Over living leaves of the fern, *Tectaria heracleifolia*, in limesinks in deepest part of Timms Hammock, north of Bauer Drive, near Homestead, Dade Co., Florida (*Schuster 33701*, Dec. 27, 1954). The type specimen has been subdivided, with portions deposited in the herbaria of the author, of Duke University, of the New York Botanical Gardens, of the Riiksmuseum of Stockholm, Sweden, and of the Hattori Botanical Laboratory.

PARATYPES:—Over a leaf of *Tectaria minima*, limesink, Timms Hammock, Florida (*Schuster 33702, c. caps.!*); in basal portion of the leaf of Sabal palmetto, deepest portion of hammock forest, Paradise Key, Everglades National Park, Dade Co., Florida (Schuster *33850b, c. caps.!*); hammock in Long Key Pineland, Section 24, Everglade Keys, Everglades National Park, Dade Co., Florida (*Schuster 22029a, p.p.*; Figs. III. and IV: 2, 5 drawn from this material).

A few sterile fragments of this species have also been found in the conduplicate basal portions of the leaf of *Serenoa repens*, the Saw Palmetto, along the Hillsborough R., in Hillsborough State Park, Florida (*Schuster 33908*). The plants have highly reduced lobes on the scattered lobulate leaves. The lobes are *ca.* 190 μ long x 90 μ wide, the lobules 120 μ long x 80 μ wide; the marginal cells of the lobe apex, and occasional keelar cells, are weakly conically elevated. These plants are of interest chiefly because of the unusual habitat in which they were found (see under *Ecology*). A second collection, from the same region (Hillsborough State Park, *Schuster 33921*) has been seen, from a decaying log. The plants also represent a rather small form, with leaves to 230 μ long. A few fragments closely matching these have also been found in Costello Hammock, Silver Palm Drive, n. of Homestead, Florida (*Schuster 22941a*, Dec. 28, 1951) which belong here on the basis of the leaf-form (lobe 190–200 μ long; lobule 110 μ long) and form of the lobule (Fig. IV: 3–4). Evans (1915) reports *A. sicaefolia* from the United States, pointing out that the plants are not well-developed and have merely acute rather than acuminate lobes. These plants (from Timms Hammock, near Bauer Drive, and from Nixon-Lewis Hammock, both in Dade Co., Florida) were found on the leaves of *Trichomanis krausii*. The plants closely agree with *A. evansii* in the acute dorsal lobes and smaller leaves (only 270 μ long). Recent study (January 1954) of the specimens, preserved in the New York Botanical Garden, has shown that they actually represent *A. evansii*.

ECOLOGY.—This extremely delicate species, smaller than the related *A. sicaefolia*, occurs usually in dense evergreen hammock forests. One of the available collections is from bark (associated with *Leptocolea cardiocarpa*, *Lejeunea* sp. and *Cololejeunea minutissima*, cf. *C. myriocarpa*. A second collection, from a decaying rather moist log (*Schuster 33921*), is associated with *Lejeunea glaucescens*, *Ceratolejeunea laete-fusca*, *Cololejeunea setiloba* and *Cheilolejeunea rigidula*. However, the species appears to be more frequently epiphyllous, occurring both on the leaves of ferns lying in limesinks (at points where the measured light

intensity is probably never above 8–10 foot candles), and on the lower leaves of the Cabbage Palm, Sabal Palmetto (where it is confined to the pleated sides of the basal portions, again in deep shade). On leaves of *Tectaria heracleifolia* the species occurs with the alga *Trentepohlia*, and such hepatics as *Rectolejeunea phyllobola*, *Caudalejeunea lehmanniana*, *Cololejeunea minutissima* cf. *myriocarpa*, *Lejeunea flava*, *Lejeunea minutiloba*, *Cololejeunea subcristata* (in *Schuster 33701–33701h*). More rarely the species grows on the leaves of the rarer *Tectaria minima*, with *Cololejeunea myriocarpa* cf. *minutissima*, *Lejeunea minutiloba*, *Lejeunea* cf. *cladogyna*, *Caudalejeunea lehmanniana* (in *Schuster 33702*). On *Sabal palmetto* leaves (in *Schuster 33850b*) are associated *Leptolejeunea elliptica*, *Leptocolea cardiocarpa*, *Diplasiolejeunea rudolphiana*, *Cololejeunea subcristata*, *Caudalejeunea lehmanniana*, and traces of the moss, *Taxithelium planum*. The latter is apparently the only report of an epiphyllous moss from the United States.[5]

The writer has searched for the plant, without avail, on the leaves of *Trichomanis*, from which it was reported (sub. *A. sicaefolia*) by Evans (1915). If *Trichomanis krausii*—or any other species of the genus, still exists in Timms Hammock, it has escaped the search of the author. Indeed, the recent history of the few remaining hammocks in the Everglade Keys (to which *A. evansii* appears to be largely confined) suggests that not only the *Trichomanis* species, but also the *Aphanolejeunea* may soon be extinct.[6]

The occurrence of traces of this species in the conduplicate folds of the bases of the leaf-blades of the saw palmetto (*Serenoa repens* [Bart.] Small) is notable, since the habitat—sandy, rather open woods along the Hillsborough R., north of Tampa, Florida, is quite exceptionally xeric. Associated were traces of a variety of other Hepaticae, some of which are only exceptionally epiphyllous: *Frullania kunzei*, *Lejeunea flava*, *L. bullata*, *Leucolejeunea conchifolia*, *Diplasiolejeunea rudolphiana*, *Leptocolea cardiocarpa*, *Cololejeunea myriocarpa* cf. *minutissima*, and brood-branches of *Leptolejeunea elliptica*! Adjacent leaves yielded also a little *Frullania obcordata*, *L. laetevirens*, and *L. longifissa*!

RELATIONSHIPS.—The species has the facies of a reduced *A. sicaefolia* (Gottsche) Evs. It agrees with it in the occasionally somewhat falcate, attenuate leaves, cell-size, perianth size, etc. Consequently, the writer at first considered the present material to belong to *A. sicaefolia* (especially since this has been reported twice from Florida). However, this conclusion appears impossible, for the following reasons:

(1) The dorsal lobe of *A. evansii* almost invariably terminates in a single apical cell, and this is never acuminate-caudate; in *A. sicaefolia*, Evans (1911) stresses that the apex is formed by a "row of two or three cells," and figures a leaftip which is acuminate-caudate (Plate 12, fig. 20). The material from El Yunque,

[5] Determination by Dr. L. E. Anderson.

[6] The only place where more than minute quantities of the *Aphanolejeunea* were found was in Timms Hammock. Here the *Aphanolejeunea* occurred scattered over some 4–5 leaves of the halberd fern, *Tectaria*, in 3 sink-holes in the hammock floor. That portion of Timms Hammock has recently been "developed", and it appears almost inevitable that the type locality will soon be destroyed.

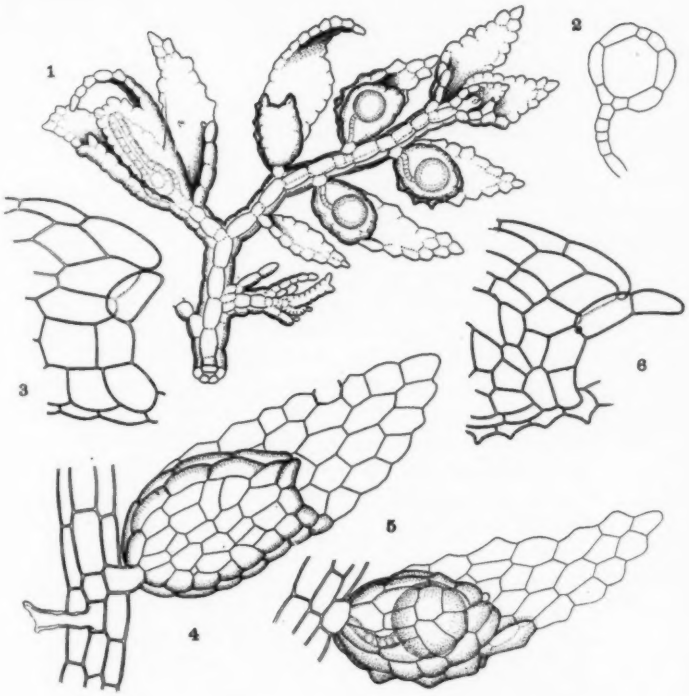

FIG. IV. *Aphanolejeunea evansii* Schuster. 1. Fertile shoot, with gynoecium and androecium (× 140); 2. Antheridium, optical section (× 325); 3. Apex of lobule (× 475); 4. Leaf, showing remnant of gemmae (× 295); 5. Male bract, postical view (× 275). *Aphanolejeunea ephemeroides*. 6. Apex of lobule, hyaline papilla indicated by stippling (× 360). (Figs. 1–2, 5, from paratype, Long Key Pineland, Florida, *Schuster 22029a*; 3–4, Costello Hammock, Florida, *Schuster 22941a*; 6, Paradise Key, Dade Co., Florida, *Schuster 22590a*).

Puerto Rico, studied by the writer, is similar, showing lobes 440 μ long x 90–100 μ wide, usually terminated by a row of 3 superimposed cells (Fig. I: 1). The long-acuminate dorsal lobe is even more highly developed on the perichaetial leaves in *A. sicaefolia*, tipped by a row of as many as 5 single, superimposed cells (see Evans, 1911, Plate 12, figs. 17, 22, 23). (2) No cells on the surface of the dorsal lobe of *A. evansii* protrude as conical extensions, although the marginal cells may be protuberant. In *A. sicaefolia* many or all of the cells of the free dorsal lobe are conically elevated. (3) The bracts in *A. evansii* possess a relatively large, narrowly lingulate lobule, rounded or bidentate at the apex, whose length is distinctly greater than one-half that of the dorsal lobe (length to 225 μ x 75 μ wide). In *A. sicaefolia* the lobule of the bracts is much smaller and described as 170 μ long x 30 μ wide and bidentate at the apex, otherwise subentire; it is de-

scribed and figured as slightly less than one-half as long as the dorsal lobe. (4) The bracts in *A. evansii* are clearly shorter than the mature perianth (Fig. IV: 2; from a plant with the sporophyte quite juvenile), and the latter, at the time of maturity of the capsule, protrudes noticeably beyond the bracts. In *A. sicaefolia*, Evans (1911) illustrates the perianth as not protruding beyond the bracts at all. (5) The perianth in *A. evansii* has the serrulate-denticulate keels angularly extended above, as shoulders; this gives the perianth an angular profile. In *A. sicaefolia* the keels are not drawn out into angles at all (compare Plate 12, figs. 17, 24, in Evans, 1911). Furthermore, the surface between the keels is quite smooth in *A. evansii*, while in *A. sicaefolia* it is usually conically denticulate as on the keels, though "sometimes smooth." The figure (Fig. III: 2–3) shows this clearly, in the material from Long Key Pineland, which is not as well-developed in certain respects as the type plants (or those from Paradise Key). In the latter two collections, the angulate shoulders are almost uniformly more strongly produced, more suberect (rather than laterally spreading), with the consequence that the perianth is truncate distally, or even has subacute to slightly acute, protruding horns. As a consequence, the condition figured (Fig. III: 2) may be regarded as the minimal development of the "horns" of the perianth. (6) The male bracts in *A. evansii* are well developed and possess discrete, inflated lobules within which the antheridia are concealed. Evans (1911) describes and illustrates the androecium of *A. sicaefolia* as formed of bracts with obsolete lobules, and reduced, short and narrow dorsal lobes, so that the bracts offer little or no protection to the antheridia. This difference appears constant and may be one of the most important characters of the new species. I have had occasion to study living material of typical *A. sicaefolia* (El Yunque, Puerto Rico; on leaves of *Hymenophyllum*). The plants of this collection have acuminate male bracts, with rudimentary lobules, as figured by Evans (1911).

When more adequate materials are known, one or two of these distinctions may prove inconstant. However, the characters of the perianth and of the male bracts have been checked on many plants and appear to be constant; they alone would warrant the taxonomic recognition of *A. evansii*.

Perhaps more closely related is *A. liliputana* (Spruce)[7] comb. n., an autoecious species which agrees with *A. evansii* in its very small leaves (*ca.* 150 μ long); however, it has obtuse leaf-lobes and an entirely smooth perianth.

Confusion of *A. evansii* with other species of the region is unlikely, except with the superficially similar (but larger, more yellowish-green) *Cololejeunea contractiloba*, which shows a dorsal lobe almost identical in shape, lanceolate-acute and terminated by a single apical cell; it also agrees in that the lobule is often about one-half the length of the lobe, and in the presence of conically elevated external cell-surfaces along the keel and leaf-margins. Several differences at once eliminate possible confusion of sterile plants: (1) the whole dorsal surface of *C. contractiloba* is coarsely papillose, due to the individually conically mamillose cells; (2) the marginal cells are only 10–12 μ on the antical margins of the lobe,

[7] *Lejeunea (Colo-Lejeunea) liliputana* Spruce, Hepat. Amaz. et And. 297, 1884.

and the median cells mostly much less elongate (15–22 μ long x 14–18 μ wide); (3) the lobule is armed with two apical teeth, separated by a discrete, open sinus.

ASEXUAL REPRODUCTION IN APHANOLEJEUNEA

Evans (1911) has described the formation of the "gemmae" or brood-bodies of the generic complex formed by *Aphanolejeunea*, *Cololejeunea*, and *Leptocolea*. He found that all of these taxa agree in producing roughly circular, discoid "gemmae", always originating on the surface of the lobe. Evans emphasized that, in contrast to such genera as *Cyclolejeunea*, these gemmae were never marginal in origin. However, this does not appear to be uniformly the case. In all the material of *Aphanolejeunea* seen by the writer the gemmae are always restricted in formation to the marginal cells of the lobe, sometimes even involving the apical cell. Such marginal gemmae are also described by Jovet-Ast (1947) for *A. longifolia* and by Allorge and Jovet-Ast (1950) for *A. teotonii*. In fact, the frequency with which gemmae are formed, together with the constancy with which they are confined to marginal cells, suggested that this restriction of the gemmae might prove of some systematic value in the separation of *Aphanolejeunea* from *Cololejeunea*.

For that reason, a study of all of our species of *Cololejeunea* was undertaken, to determine the mode of origin and restriction of the gemmae in that genus. Study of all of the species has shown that some species have the gemmae apparently uniformly confined to the postical surface of the lobes (*C. biddlecomiae*), others may have them developed both on the postical face as well as from marginal cells (*C. minutissima*), while still others (*C. diaphana*, *C. setiloba*) may develop them exclusively from the antical leaf surface. This study shows that the modes of origin of the gemmae in *Cololejeunea* are much more diverse than had been suspected, and that marginal gemmae may also be produced in *Cololejeunea*. As a consequence, the restriction of gemmae to marginal cells in *Aphanolejeunea* acquires only a limited significance in the separation of that genus from *Cololejeunea*. However, no species of *Cololejeunea* has been found in which the gemmae originate *only* from marginal cells.

The pattern of development of the gemmae in *Aphanolejeunea* appears to be quite constant, with the following sequence: (1) a marginal cell swells and becomes turgid and opaque (Fig. III: 4, upper right) and protrudes somewhat beyond the margin of the leaf; (2) a vertical division occurs, parallel to the edge of the lobe; the proximal cell becomes the stalk cell, the distal develops eventually into the gemma; (3) a vertical division of the distal (and sometimes also of the basal) cell occurs, at right angles to the first division and to the leaf-margin (Fig. III: 4, lower left); (4) the distal two cells each undergo a division on a horizontal plane, resulting in a disc of four cells, lying at right angles to the leaf-surface; the basal cell (which may still be undivided, or may be divided into two cells) does not appear to undergo further divisions; (5) the distal 4 cells each divide into an external and a central cell, resulting in a plate of 8 cells, 4 inner and 4 peripheral; (6) two of the quadrants exhibit each a division of the external cell, while the inner cell undergoes no further division, resulting in 3-celled

quadrants; (7) the two other quadrants each show one division of the inner cell, and a division of the outer cell into three cells, resulting in 5-celled quadrants; (8) three of the marginal cells of the resulting 16-celled plate swell, developing eventually into slime-cells which serve to secrete a mucilaginous substance that causes the absisced gemma to adhere to the substrate it falls on.

One consequence of the marginal mode of development of the gemmae is that the mature gemmae always appear in lateral profile, when still *in situ* on the parent leaf (Fig. II: 1), since they develop at right angles to the leaf-surface. When mature, dehiscence occurs by rupture of the basal cell, as is evident from Fig. IV: 4.

LEPTOCOLEA (SPRUCE) EVANS

Lejeunea subg. *Colo-Lejeunea*, sectio *Leptocolea* Spruce, Hep. Amaz. et And., TRANS. Proc. Bot. Soc. Edinburgh **15:** 292, 1884.

Cololejeunea subg. *Leptocolea* Schiffen., *in* Engler & Prantl, Nat. Pflanzenfam. **1**(3): 122, 1895.

Leptocolea Evans, Bull. Torr. Bot. Club **38:** 261, 1911.

Plants pale green to yellowish-green, on drying often discolored, *usually less minute and firmer than Cololejeunea*. Stems extremely slender, showing more or less zig-zag curvature, closely attached to the substrate by small fascicles of short rhizoids, originating below the insertion of each leaf; *stems with 5–6 rows of cortical cells that are not, or barely, larger than the single row of medullary cells*, the ventral merophytes formed by 1 or 2 cell-rows, never developing underleaves. Leaves extremely variable in form in different species, but *isomorphic in any one species, frequently rounded distally*, distant to slightly imbricate; lobe flat or nearly so, its cells smooth (or more rarely tuberculate); *lobule identical with that of Cololejeunea*,[6] inflated, typically with 2 teeth near apex, the proximal smaller and usually obtuse, the *distal large, 2-celled and with hyaline papilla essentially near proximal base, but sometimes entally displaced* and nearly midway between a proximal and distal position (sometimes apical tooth enlarged and elaborated, apendiculate, then with hyaline papilla at its apex); lobules typically smaller than in *Cololejeunea*, averaging 0.3–0.5 × the length of the lobe; stylus reduced to a hyaline papilla or a slender filament several cells long. Cells variable, usually delicate and with small trigones (and sometimes small but nodular intermediate thickenings); ocelli sometimes present (not in ours); *marginal cells very frequently differentiated as a hyaline border of dead cells, and/or a group of hyaline, finger-like terminal cells*; oil-bodies several per cell (usually 3–15), formed of discrete, individually protruding segments, *ca.* 2–4.5 x 3–7 μ. Asexual reproduction by means of discoid brood bodies, occurring on the lobe surfaces, *never marginally*; brood-bodies relatively large and complex (formed of about 20–40 cells).

Autoecious, more rarely paroecious (or synoecious), or dioecious. Female inflorescence often on a short, sometimes on an elongate shoot, innovating on one or both sides, one or both innovations often again floriferous; ♀ *bracts like leaves, often slightly smaller* and with plane lobules (particularly when not bearing antheridia and when situated above an innovation). *Perianth* somewhat to strongly dorsiventrally *compressed, the antical keel low and short (or absent)*, the lateral keels sharp, *sometimes dilated above and wing-like or inflated* (then produced as low horns or rounded auricles, the apex obcordate and the beak becoming

[6] In many exotic species the lobule deviates considerably. It may be reduced to a small basal fold bearing two approximated cilia-like teeth; it may bear only a single apical tooth, with the hyaline papilla at its apex; or it may be variable within the same species, bearing alternately 1 or 2 apical teeth.

situated in a more or less deep sinus); ventral keels either sharp and discrete, sometimes weakly horned, or reduced to a common, median, low, more or less biangulate keel, or absent. Male bracts variable, often similar to vegetative leaves (at other times smaller, more nearly imbricate, and forming a discrete spike of 3–6 or more pairs of reduced bracts). Sporophyte as in *Cololejeunea*.

TYPE: *Leptocolea micrandroecia* (Spruce) Evs. (= *Lejeunea* (*Cololejeunea*) *micrandroecia* Spruce, Hep. Amaz. et And. 298, 1884).

Leptocolea is a rather large genus, with over 40 species (including those listed in Stephani and those described subsequently; of these perhaps 20% will prove synonymous). Only a single species of this largely tropical genus extends into our region.

The genus *Leptocolea* differs, in the final analysis, from *Cololejeunea* only in the dorsiventral compression of the perianth (with consequent reduction or even obsolescence of the antical keel). In *Cololejeunea* the equally 5-carinate perianth is usually erect in position, standing at right angles to the vegetative plant and to the substrate; in *Leptocolea* the perianth usually lies nearly in the same plane as the rest of the plant. Although our species (*L. cardiocarpa*) has a relatively unmodified perianth, differing from that of *Cololejeunea* only in the somewhat compressed form and weaker development of the antical carina, exotic species often show highly modified perianths. For instance, the Hawaiian *Leptocolea obcordata* (= *Lejeunea obcordata* Aust., Bot. Gaz. **1:** 36, 1876) and *Leptocolea ceatocarpa* (= *Lejeunea ceatocarpa* Angstr., Öfversigt af Kongl. Vetensk. Akad. Förhand. **29:** 27, 1872) both show a strongly compressed perianth, without any trace of antical keel (and with the two postical keels fused to form a weak and low rounded keel), but with the dilated lateral keels extended upward as flat wings, resulting in a deep distal sinus in which the small beak is situated. Similarly, the distinctive *Leptocolea ovalifolia* (= *Cololejeunea ovalifolia* Evans, Trans. Conn. Acad. Sci. **10:** 450, 1902), though not distinctly compressed, has the antical keel absent, while the lateral and postical keels are produced as discrete, subacute horns. The recently described *Cololejeunea cornuta* E. W. Jones, is similarly armed with four horns, which extend well beyond the perianth-beak.

The vegetative features of *Leptocolea* agree thoroughly with those of *Cololejeunea*. As a consequence, sterile plants often can scarcely be separated. This very close similarity is best illustrated by comparing *C. subcristata* with *Leptocolea cardiocarpa*. The separation of two genera, on the basis of the compression (or lack of compression) of the perianth appears to be essentially artificial. This opinion was also recently expressed by E. W. Jones (1954, p. 408) who stated: "even typical species of *Leptocolea* . . . may have perianths with a low but sharp dorsal keel, and with distinct ventral keels when young, and conversely many species here classed as *Cololejeunea* have perianths which are either more or less compressed, especially when young, or which are plane and unkeeled dorsally and inflated and scarcely keeled ventrally. So far as there is any distinctive feature in the *Leptocolea* perianth, it lies in the presence of broad lateral wings rather than in compression or in the absence of a dorsal keel. A more natural

subdivision into several genera could perhaps be based on the structure of the lobule which is certainly of considerable systematic importance. . . ."

The species of *Leptocolea*, as those of *Cololejeunea* and *Aphanolejeunea*, are almost always epiphytic, occurring often indiscriminately on the bark of trees or on living leaves. Often the same species may show a wide range of variation in "host" species, and may occur on both the leaves and twigs, as well as trunks, of the same tree or shrub. The ability to undergo ecesis rapidly in such temporary habitats as on living evergreen leaves appears to hinge on a rapid rate of growth, and the ability to produce discoid "gemmae" freely. In extreme cases (*Leptocolea cardiocarpa*) ecesis may take place on the leaves of such semi-evergreen trees as *Quercus nigra*, the Water Oak. Such cases are surely exceptional. In the United States, *Leptocolea cardiocarpa*, our only representative of the genus, is equally "at home" on the surfaces of a wide variety of leaves, and on the bark of an extensive series of trees and shrubs.

Leptocolea cardiocarpa (Nees and Mont.) Evs.

Lejeunea cardiocarpa Mont. *in* Ramon de la Sagra, Hist. Fis. Pol. y Natur. Cuba **9:** 476, pl. 18, f. 4, 1845.

Lejeunea jooriana Aust., Bull. Torrey Bot. Club **6:** 20, 1875.

Lejeunea (subg. *Colo-Lejeunea*, sect. *Leptocolea*) *cardiocarpa* Spruce, Trans. Proc. Bot. Soc. Edinburgh **15:** 300, 1884.

Lejeunea (*Colo-Lejeunea*) *jooriana* Steph., Bot. Gaz. **17:** 171, 1892.

Cololejeunea jooriana Evans, Mem. Torrey Bot. Club **8:** 173, pl. 22, figs. 9–20, 1902.

Leptocolea cardiocarpa Evans, Bull. Torrey Bot. Club **38:** 268, p. 12, figs. 1–3, 1911.

Leptocolea jooriana Evans, Bull. Torrey Bot. Club **38:** 270, f. 2B, 1911.

In thin, closely appressed, flat, yellow-green, usually small patches, freely branching, closely adnate to substrate. Shoots (675) 800–1200 μ wide when mature. Stems with weak but discrete zigzag or sigmoid, slender (40–50 μ wide), producing small fascicles of rhizoids near (and slightly below) each leaf. *Leaves flat when dry or wet,* closely adnate to substrate, *ovate to ovate-triangular,* widely spreading (55–75°), somewhat imbricate, when well developed extending, at dorsal base, across and often slightly beyond the stem (which is then totally hidden from above). Lobe with antical and postical margins faintly convex, nearly straight, convergent, the *lobe somewhat pointed* (but usually obtuse to narrowly rounded at the very tip), *ca.* (305) 450–560 (750) μ long x (210) 320–375 (450) μ wide, strongly narrowed at base, the *apex of mature and well-developed leaves usually tipped with a group of hyaline, finger-like dead cells* (these *ca.* 12–15 x 18–30 μ); anterior margin of lobe, from near base to near apex occasionally margined with thin-walled, hyaline, rectangular to quadrate cells, forming a discrete border. Lobule inflated, ovate to broadly ellipsoidal, strongly constricted at base, averaging 0.4–0.5 as long as lobe (*ca.* 160 μ long x 120 μ wide to 250 μ long x 150 μ wide), its keel moderately strongly convex, barely crenulate due to the convex cells; keel nearly continuous with posterior leaf-margin; *apex of lobule marked by on obtuse, 1-celled tooth: midway between this and the keel is situated an elongate, 2-celled apical tooth.* Cells all of one type, thin-walled and with minute to small trigones, often with faint to rather distinct intermediate nodular thickenings, *ca.* 13–15 μ near apex and on margin, *ca.* 16–17 x 18–23 μ medially; oil-bodies typically 3–7 per cell and fusiform to narrowly ellipsoidal or sublinear (then mostly of 2–3 rows of discrete, individually protruding segments), *ca.* 1.5–2.2 x 3–5, occasionally to 6–8 μ; occasional cells with 7–15 (18) smaller, usually subspherical, fewer-segmented oil-bodies, *ca.* 2–3 μ to 2.5–3 x 4 μ; in a few cells occasional oil-bodies of only 2–5 coarse segments. Asexual reproduction by means of discoid gemmae, produced from the lower surface of the lobe; gemmae unspecialized, of 25–28 cells (margin usually of 14 cells), broadly oval, *ca.* 60 x 70 μ.

Autoecious or paroecious. Male bracts (when on a separate branch) constituting a short branch with small, reduced bracts (in 3–7 or up to 15 pairs) or situated below gynoecium, then like unmodified leaves in form; the androecium then often of only 1–3 pairs of bracts virtually indistinguishable from vegetative leaves (androecium then 650–675 μ wide); bracts mon- or diandrous, usually without the apical hyaline cells. Male bracts, when situated below perianth, either unmodified from vegetative leaves (when situated in leaves below innovations, below the immediate perichaetial bracts), or formed by perichaetial bracts. Perichaetial bracts usually slightly smaller than leaves, often dimorphic: the one opposite the subfloral innovation with a large, inflated lobule, often widely spreading and obtuse at apex, with margins undentate; the one situated immediately above the subfloral innovation usually with lobule narrower and acute, often bearing 1–several small teeth with hyaline papillae at apices; lobes of perichaetial bracts similar to leaves, to 480–500 μ long (averaging somewhat smaller than normal vegetative leaves), their apices terminated by a group of papilloid hyaline cells (as on vegetative leaves). Perianth situated on a main branch or occasionally more or less elongate lateral branch, subtended inevitably by a single subfloral innovation (which may be almost immediately floriferous), 360–400 μ wide x 500–550 μ long, obovate to obpyriform in outline, with two sharp and barely crenulate lateral keels, and two less sharp barely crenulate postical keels (extending down to within the basal one-half to one-third of perianth), rather strongly dorsiventrally compressed and *with antical keel sharp but weak and shorter than postical*; perianth subtruncate at apex, except for the short and broad beak.

TYPE: Cuba (*L. cardiocarpa*); the type of *L. jooriana* from Louisiana.

DISTRIBUTION.—A species of nearly strictly Coastal Plain distribution, with exceptional occurrences in the outer edge of the Piedmont (as far north as North Carolina). Limited to the Lower Austral, Subtropical and Tropical Regions, extending from northeastern North Carolina to the southern tip of Florida, westward at least to Louisiana.

NORTH CAROLINA: Washington Co. (near Roper, *RMS 28356*,[9] *28369*, *28364*); Dare Co. (Kitty Hawk, *RMS 28377a*; near Collington, *RMS 28310*, *28331a*); Columbus Co. (e. of Whiteville, *RMS 29227*); Durham Co. (Duke Campus, *Mitchell 29486*); Brunswick Co. (Holden Beach, *Blomquist 7349*); Carteret Co. (Harkers I., *Blomquist 11311*; Beaufort, *Blomquist 11310*; Shackleford Bank, *Johnson*; Salter Path, Bogue Sound, *RMS 33641*); Pender Co. (near N.E. Cape Fear R., 10–12 mi. n. of Wilmington, *RMS 33646*). GEORGIA: Liberty Co. (n. of Midway, *RMS 31415* [plate], *RMS 31416*); Thomas Co. (Thomasville, *Brown 8050*). MISSISSIPPI: Harrison Co. (w. of Biloxi, *RMS 20392b*, *20405*, *20400*, *20397*; 19 mi. n. of Gulfport, *RMS 19750d* [plate]; 5–7 mi. s. of Ramsey Springs, *RMS 27761a*); Jackson Co. (3 mi. s.e. of Ocean Springs, *RMS 19232* [plate]). LOUISIANA: type of *L. jooriana*. ALABAMA: near Chunchulla, Mobile Co. (*Schuster*). FLORIDA: Seminole Co. (Sanford, Rapp). Lake Co. (Alexander Springs, Ocala Ntl. Forest *RMS 33316b*). Marion Co. (Juniper Springs, Ocala Ntl. Forest, *RMS 31915a*, *31915b*, *33459*, *31942*, *31938*, *31944*; Ocala Ntl. Forest, 5–6 miles e. of Astor Park, *RMS 31916*). Nassau Co. (Ft. Clinch State Park, *RMS 31989*); Clay Co. (Gold Head Branch State Park, *RMS 31401*); Hernando Co. (n.w. of Brooksville, *RMS 22086a*). Collier Co. (Collier-Seminole St. Park, *RMS 26140b*). Dade Co. (Hammock on Loveland Rd., 5–6 mi. w. of Florida City, *RMS 22680*, *22676*, *22666*, etc.; Paradise Key, Everglades Ntl. Park, *RMS 22740b*, *19912*, *22590*, *22914*, *33850a*, Hammock, section 24, Long Key Pineland, *RMS 22053*, *22047b*, *22010a*, *22038a* [plate]); Monroe Co. (Rte. 94, 1 mi. w. of Dade Co. line, *Schuster*; Rte. 94, 3 mi. s. of Monroe Station, *RMS 33999*); Polk Co. (Bok Tower, *Fisher 130*); Hillsborough Co. (Hillsborough R. State Park, *RMS 33908*, *33909d*).

[9] In this and succeeding papers of this series, routine citations of specimens collected by the author have the author's name abbreviated to the initials, *RMS*.

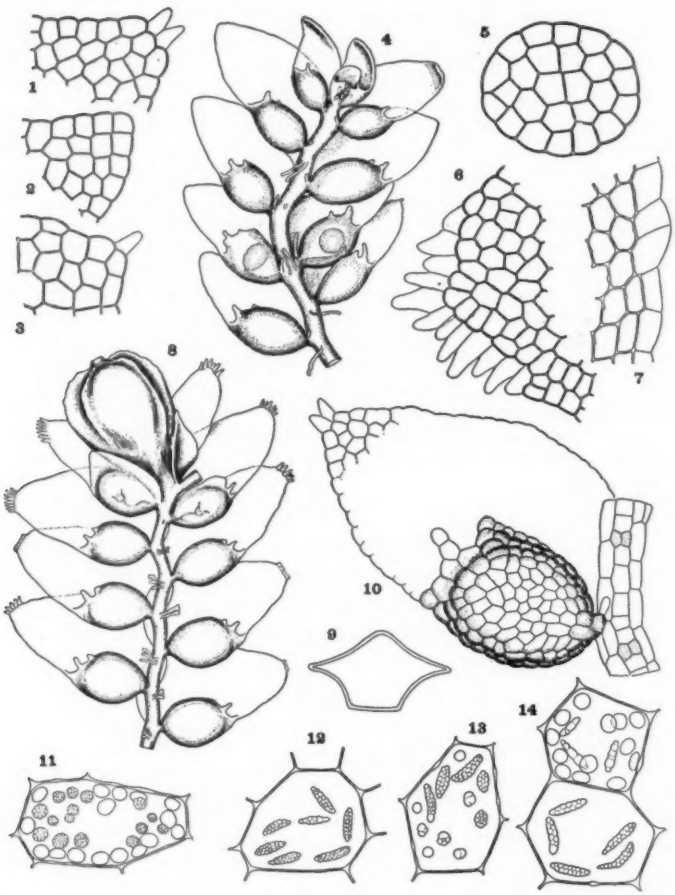

FIG. V. *Leptocolea cardiocarpa*. (N. et M.) Evs. 1–3. Apices of leaf-lobes, of a weak phase (× 250); 4. Male branch (× 55); 5. Discoid "gemma" (× 340); 6. Apex of well-developed leaf, showing numerous hyaline papillae (× 295); 7. Anterior margin of an epiphyllous phase, showing border of hyaline, dead cells (× 295); 8. Paroecious shoot, the innovation cut off at base (× 40); 9. Perianth cross-section, median (× 50); 10. Leaf of small, xeromorphic phase from bark (× 175); 11–14. Median cells of leaves, showing oil-bodies, and in two cells, chloroplasts (11, × 775; 12, × 790; 13, × 730; 14, × 875). (Figs. 1–4, 10, Ocean Springs, Miss., *Schuster*; 5, Florida City, *Schuster 22680;* 6–7, Paradise Key, Florida, *Schuster 21481, 22590;* 8–9, Georgia, *Schuster 31415;* 11–14, from Schuster and Hattori, 1954).

Also known from Bermuda, Cuba (type), Jamaica, Puerto Rico, and other islands of the Antilles; doubtfully known from Mexico (Gottsche, 1867), and extending south to South America (*fide* Stephani).

ECOLOGY: Occurring nearly indifferently on the bark of trees (usually on

smooth-barked trees) and on the leaves of a variety of plants (in tropical and subtropical regions). When epiphyllous only on the leaves of broad-leaved evergreens (among them: *Laurocerasus caroliniana*, *Eugenia* sp., *Dipholis salicifolia*, *Coccolobis laurifolia*, *Ocotea catesbyana*, *Ilex cassine*, *Persea borbonia*, *Magnolia grandiflora*, *Desmothamnus* sp., *Gordonia lasianthus*, northward rarely on leaves of *Kalmia latifolia* and *Quercus nigra*; more rarely on the fronds of the palms *Raphidophyllum hystrix*, *Sabal palmetto* and *Serenoa repens*). On leaves evidently occurring only in the most humid and deepest hammock forests, in usually dense shade. Associated on leaves of the *Dicotyledonae* are usually *Leptolejeunea elliptica*, *Cololejeunea* cf. *myriocarpa*, or *Caudalejeunea lehmanniana*, rarely *Drepanolejeunea bidens*. On the leaves of palms, a wider series of associated species are found, including most often *Leptolejeunea elliptica*, *Aphanolejeunea evansii*, *Caudalejeunea lehmanniana*, *Cololejeunea subcristata*, and *Diplasiolejeunea rudolphiana* (on *Sabal palmetto* leaves, *RMS 33850–33850e*, associated with the moss *Taxithelium planum*). In most exposed sites, on the leaves of both the Sabal as well as on those of *Serenoa repens*, the Saw Palmetto, are sometimes associated traces of even a longer series of species, among them: *Diplasiolejeunea rudolphiana*, *Aphanolejeunea evansii*, *Cololejeunea* cf. *myriocarpa*, *Lejeunea bullata*, *flava* and *longifissa*, *Leucolejeunea conchifolia*, as well as *Frullania kunzei* and *obcordata*.

More frequently on bark of a wide range of trees, in our region, including *Quercus virginiana*, *Q. chapmani*, *Q. myrtifolia*, *Magnolia grandiflora*, *M. virginiana*, *Ostrya virginiana*, *Myrica cerifera*, *Planera aquatica*, *Acer rubrum*, *Carya* sp., *Salix nigra*, *Fagus grandifolia*, *Nyssa sylvatica*, *Ilex opaca* in non-tropical areas; *Erythrina herbacea*, *Ocotea catesbyana*, *Elaphrium simaruba*, *Gordonia lasianthus*, *Guettarda scabra*, *Baccharis halimifolia*, *Desmothamnus lucidus*, Royal Palm (*Royostonea regia*), *Alvaradoa amorphoides*, *Metopium toxiferum*, and many other trees in tropical and sub-tropical regions. As on leaves, the bark populations are strictly pioneer in nature, forming tightly appressed, usually small yellowish-green patches less than 1 cm. in diameter—the small patches rarely confluent. The most frequenty consociated species on bark are *Frullania obcordata*, *F. squarrosa*, *F. donellii*, *Leucolejeunea unciloba*, *Radula caloosiensis*, *Rectolejeunea maxonii*, *Cololejeunea minuttissima*, *Lejeunea flava*, *Drepanolejeunea bidens*, *Microlejeunea laetevirens* and *bullata*, *Euosmolejeunea rigidula*, in non-tropical regions; in tropical areas the above species, as well as *Caudalejeunea lehmanniana*, *Cololejeunea subcristata*, *Mastigolejeunea auriculata*, *Leptolejeunea elliptica*, *Diplasiolejeunea rudolphiana*, *Cheilolejeunea decidua*, *Frullania riojaneirensis*, and *Aphanolejeunea evansii*, and other species are frequently associated.

The species occurs on bark under both extreme xeric conditions, as in the Sand Pine-Scrub Oak scrub of central Florida (there on bark of *Quercus chapmani*, *Q. virginiana gemminata*, *Q. myrtifolia*, associated with *Frullania kunzei* and *Leucolejeunea conchifolia*, etc.), as well as in shaded damp hammock forests. It does not appear able to compete with larger, coarses species; as a consequence it begins to disappear when the more robust species undergo ecesis, standing thus in a direct successional relationship with these.

Differentiation:—Leptocolea cardiocarpa is usually easily identified by the distal group of finger-like, dead, hyaline cells of the leaves. When these can be found, they offer an absolute criterion for the identification of the species, since no other Lejeuneaceae of our area possess such cells. Unfortunately, these are often poorly or not developed (Fig. V: 1–4) and may be reduced to 1 or 2 papillae on occasional leaves. If these occasional distal papillae are overlooked, the plant may well be mistaken for the otherwise exceedingly similar *Cololejeunea subcristata*.

The species is quite variable, especially as regards the spatial relationships of androecia and gynoecia, and as regards size, leaf-shape and development of hyaline, dead cells. Perhaps the most luxuriantly developed plants are those of the deep hammocks of southern Florida, of living leaves. Such plants almost always show a conspicuous development of the finger-like glistening hyaline cells of the leaf-tips, and often show a more or less extensive border, along the anterior leaf-margin, of quadrate hyaline, dead cells (Fig. V: 7). This border may be one or several cells wide, and may extend for most of the anterior leaf-margin. Although frequently found in epiphyllous phases, it has not been found in corticolous modifications of the species. It has not been previously noted in this species, although a similar, more extensively developed border characterizes the Puerto Rican *Leptocolea planifolia* Evs.

A NEW GENUS AND FAMILY OF THE ACTINOMYCETALES, WITH A REVISION OF THE GENUS ACTINOPLANES*

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When the genus *Actinoplanes* was first described in 1950, there were included in it two collections (D.S. 22 and 34) of a species which has sporangia, but non-motile spores. Since then, we have found two additional species with sporangia and non-motile spores. This group of three species is so different from *Actinoplanes* in spore behavior and growth characters that it is being described here as a new genus. The sporangial character, however, allies it closely to *Actinoplanes*. Under certain conditions of culture, the species of this new genus form an aerial mycelium which resembles that in *Streptomyces*, and one species, in addition to sporangia, forms coils on the aerial hyphae. Because of this resemblance to *Streptomyces*, the new genus is named *Streptosporangium*.

These two genera, *Actinoplanes* and *Streptosporangium*, while clearly belonging in the Order Actinomycetales, are so distinct from the other three families that it

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seems necessary to establish a new one for them. The key below, revised from Bergey's Manual (1948) to include the new family, points out how the families are distinguished. A description of the new family follows the key.

KEY TO FAMILIES OF ORDER ACTINOMYCETALES

I. Mycelium rudimentary or absent, no spores formed.
Family I. Mycobacteriaceae.

II. True mycelium produced, spores formed but not in sporangia.
A. Spores formed by fragmentation of mycelium.
Family II. Actinomycetaceae.
B. Vegetative mycelium normally remains undivided.
Family III. Streptomycetaceae.

III. True mycelium produced as in "B" above, spores formed in sporangia.
Family IV. Actinosporangiaceae.

ACTINOSPORANGIACEAE Fam. nov.

Actino, short form for Actinomycetes, ray fungi. *Sporangium*, from Latin, a spore case.

Vegetative mycelium formed in water on a variety of plant and animal parts, usually inconspicuous; aerial mycelium lacking as a rule, formed in certain species and then much as in *Streptomyces*. Reproduction by spores formed in sporangia, the spores in *Actinoplanes* with flagella and motile, in *Streptosporangium* without flagella and non-motile; conidia formed in many species. Culturable on a variety of natural and artificial media and then resembling, in vegetative characters, certain species of *Nocardia*, *Micromonospora*, or *Streptomyces*.

Widely distributed in soil and fresh water.

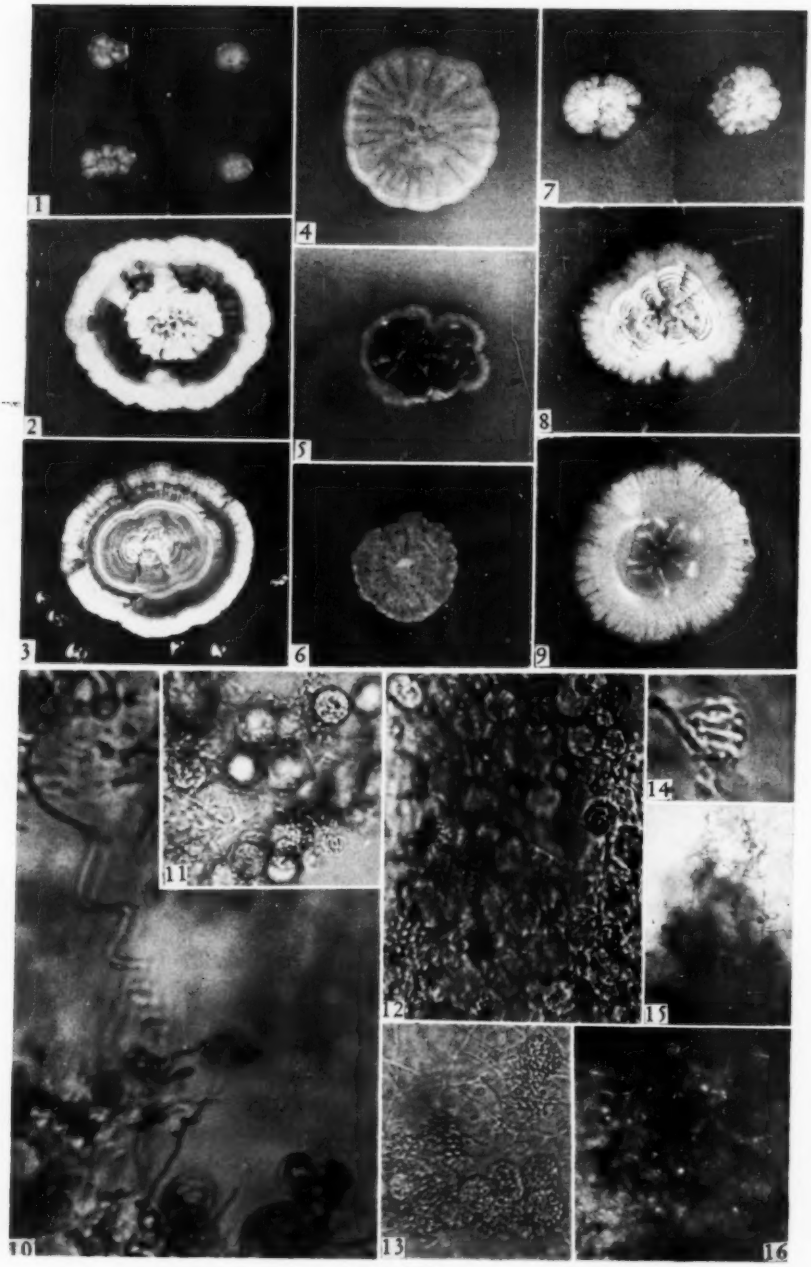
Actinoplanes is designated as the type genus of the new family since it is far and away the commoner of the two and much the more typical and interesting. It represents, furthermore, the culmination in this line of development whereas *Streptosporangium* is an intermediate genus between *Streptomyces* and *Actinoplanes*.

Streptosporangium gen. nov.

"*Strepto*" from Greek meaning "coiled" is combined with "*sporangium*" to mean "Streptomyces-like" but with sporangia.

On sterilized leaves of *Paspalum* grass in water forming an inconspicuous mycelium which overgrows the leaves, and an aerial mycelium which grows in scattered or concentrically arranged tufts; aerial mycelium white to pinkish on leaves; hyphae much branched, sparingly septate and about 0.5–1.2 μ in diameter. Sporangia formed abundantly on leaves on the aerial mycelium; spores abundant in sporangia, without flagella and non-motile. Growth poor to good on a variety of semi-solid media; aerial mycelium absent to abundant; sporangia and conidia formed on some nutrient agars.

This genus is represented by four isolates, representing three distinct species. Two of the species were isolated from soil by the soil dilution method used by



FIGS. 1–16

Jensen (Proc. Linn. Soc. N. S. Wales **55,** 1930–31, 238), and the third one was isolated from dog dung collected in New York City by Dr. L. S. Olive.

For a comparison of this genus with *Actinoplanes* see under the latter.

Streptosporangium roseum sp. nov.

Roseum, from the pinkish color on potato dextrose agar.

On sterile leaves, either in soil water or on damp sterile soil, forming a vegetative mycelium which spreads over the surface of the leaf, not penetrating or decolorizing it, also spreading over the soil; and an aerial reproductive mycelium which is white at first but soon changes to pale pink. Aerial mycelium appearing as single hyphae or as minute tufts which grow to form mounds up to 2 mm. across, arranged more or less in concentric circles; mounds usually becoming minutely pock-marked. Sporangia first appearing on scattered single hyphae, apical on the main thread or on short, lateral branches, a few to many sporangia on one hypha; formed in the hyphal tufts and mounds until the latter may be almost solid masses of sporangia; white in small groups, pink in large masses, spherical, 7–19 μ in diameter on leaves, most 8–9 μ. Shortly after their formation, spores visible as a single coil in each sporangium; when completely formed, irregularly arranged. Immersion of the mature sporangium in water bringing about the swelling of an intersporal substance, causing the wall and the spores to push out on one side, forming a cone-shaped projection about half as long as the diameter of the sporangium. Spores forcibly ejected through an opening in the cone; spherical, 1.8–2.0 μ in diameter, with one shiny globule, non-motile. Sporangial wall persistent for several hours after spore discharge. In addition to sporangia, conidia formed in coils somewhat as in *Streptomyces*, though the coils are much less conspicuous.

Czapek agar: Growth fair, about 0.7–1.2 cm. in diameter after 6 weeks; usually flat, level with agar surface; concentric zonation distinct or absent; central region usually compact with a broad fringed border and a tasseled edge. Surface glossy or powdery. Color usually white, sometimes pinkish-buff or cream-buff. Sporangia absent to fairly abundant, always formed some distance

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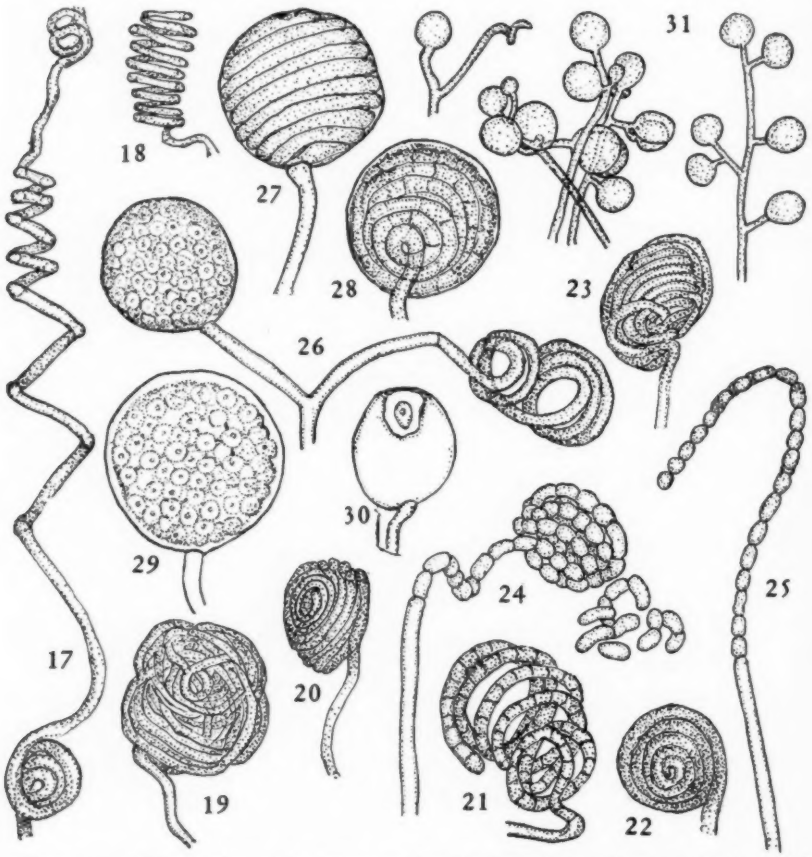
FIGS. 1–16. *Streptosporangium roseum*. FIGS. 1–9. On various agars. All ×1½ except fig. 4, which is about ×2. FIG. 1. On Krainsky's dextrose asparagin agar Dec. 6, 1954; photographed Jan. 24, 1955. Very little aerial growth, no sporangia. FIG. 2. On Emerson's agar Dec. 6, 1954; photographed Jan. 24, 1955. Sporangia abundant in pinkish-white center. FIG. 3. On Magnolia soil water peptone Czapek agar Feb. 13, 1953; photographed April 24, 1953. Note concentric rings. On the broad white margin are vast numbers of sporangia. FIG. 4. On nutrient agar Feb. 27, 1954; photographed April 23, 1954. Surface usually glossy, color cream buff, no sporangia. FIG. 5. On nutrient agar March 20, 1953; photographed May 6, 1953. Surface glossy, color buffy brown, no sporangia. FIG. 6. On nutrient agar Dec. 16, 1953; photographed Feb. 12, 1954. No sporangia. Color as in fig. 4, but surface irregularly ridged. FIG. 7. On Czapek agar March 21, 1953; photographed May 6, 1953. Center with aerial hyphae and sporangia. FIG. 8. On peptone Czapek agar March 20, 1953; photographed May 6, 1953. Center with concentric zones of aerial hyphae, no sporangia. FIG. 9. On peptone Czapek agar July 8, 1954; photographed August 25, 1954. Surface glossy, no sporangia.

FIGS. 10–16. Photographs of sporangia and coils. FIG. 10. One long open coil, and several compact ones at lower edge of photo. On Czapek agar. ×1400. FIGS. 11–13. Sporangia, coils and discharged spores; figs. 11, 13, on potato dextrose agar, ×600; fig. 12, on Czapek agar, ×700. FIG. 14. Sporangium with distinct wall and coiled hypha inside. On Czapek agar. ×1400. FIG. 15. Part of a tuft of aerial hyphae with sporangia, photographed with transmitted light. On potato dextrose agar. ×100. FIG. 16. As above, photographed with direct light. ×100.

above the surface of the agar. In some cultures, coils formed, which break up into conidia as in *Streptomyces*. Palisades absent.

Peptone Czapek agar: Growth good, about 1.5–2.0 cm. in diameter after 6 weeks; flat or with a few low radial or irregular ridges and grooves; margin fringed or entire; aerial hyphae often formed in white concentric rings, sometimes as a white border, and sometimes giving a powdery appearance to the normally glossy surface. Color olive buff to deep olive buff. Sporangia very rare.

Potato dextrose agar: Growth usually good, 1.0–1.8 cm. in diameter after 2 months; center elevated with irregular bumps and ridges; margin flat and even with surface of agar. Color at first creamy, becoming tawny and then Carob brown or Kaiser brown, after which white floccose spots of hyphae appear, spreading usually to cover the entire culture. Sporangia usually formed in vast



FIGS. 17–31. *Streptosporangium roseum*, on Czapek agar. All ×2450 except fig. 31, which is ×1330. FIGS. 17–25. Coils, spores forming in figs. 24 and 25. FIG. 26. Sporangium and coil on same hypha. FIGS. 27–29. Sporangia, fig. 29 with spores formed. FIG. 30. Sporangium with all spores discharged except one. FIG. 31. Clusters of sporangia.

numbers, the white areas becoming rosy pink as the sporangia mature; the pinkish areas frequently minutely pocked. Surface moist at first, appearing dry and floccose as aerial hyphae and sporangia are formed. Agar colored reddish brown with a vinaceous tinge.

Nutrient agar: Growth fair, 0.7–1.3 cm. in diameter after 2 months; central region elevated into irregular ridges which merge, towards the outside, into radial ridges and grooves sloping abruptly to the narrow, flat border; margin lobed. Color usually cream-buff, rarely buffy-brown. Surface usually glossy, sometimes powdery with aerial hyphae which may be united to form many upright fascicles. Sporangia absent.

Krainsky's dextrose asparagin agar: Growth poor, 0.3–0.7 cm. in diameter; slightly elevated and minutely ridged, sloping to the fimbriate margin. Surface of the central region minutely powdery with aerial hyphae. Color white. Sporangia absent.

Emerson's agar (YpSs): Growth good, about 2 cm. in diameter after 6 weeks, composed of a whitish central area, 4–6 mm. wide, made up of elevated, irregular bumps and ridges, which abruptly changes into radial ridges and grooves, sloping down to a flat, white border, 1–2 mm. wide, and composed of minute concentric circles of white hyphae. Ridges and grooves vinaceous brown, sometimes covered with a whitish down. Margin smooth or scalloped, ending abruptly. Surface dry. Sporangia formed abundantly first in the center as the white changes to pink. Agar colored pale vinaceous brown.

Habitat: Vegetable garden soil.

With the removal from the genus *Actinoplanes* of the species on which *Streptosporangium* is based, it is necessary to revise the description of the latter genus.

Actinoplanes Couch (*Actinoplanes*, A New Genus of the Actinomycetales. Jour. Elisha Mitchell Sci. Soc. **66,** 1950, 87; *Myceliochytrium fulgens* Johanson, in part, Torreya **45,** 1945, 104).

On sterilized leaves in water forming a very inconspicuous mycelium which branches throughout the leaf tissue, the external hyphae scattered or in tufts on the leaf surface and forming a fringe around the edge of the leaf; aerial mycelium lacking or sparingly formed as a rule; mycelium usually pinkish to reddish, sometimes hyaline, on leaves, frequently decolorizing the green leaf and giving it a pinkish or reddish color; hyphae slightly to considerably branched, irregularly coiled, twisted or straight, sparingly septate, 0.2–2.6 μ in diameter. Sporangia usually abundant on leaves, formed only when the leaf is at or close to the surface of the water, i.e. formed typically only in air, and appearing black under the low power of the microscope, owing to refraction; of varied sizes and shapes. Spores in coils, nearly straight chains, or irregularly arranged, in sporangia; 1–1.5 μ in diam., globose or subglobose, usually slightly angular, with one to several shiny bodies, with several polar flagella, and motile; germination by a minute germ tube which branches to form a mycelium. Sporangial wall evanescent or persistent.

On various nutrient agars forming, usually, a brilliantly colored, toughish to pasty growth; surface very variable: smooth and even with the agar or elevated, bumpy, convoluted, ridged, folded, cracked, etc., usually moist and shiny, rarely pulverulent. Hyphae of two more or less distinct forms, the submerged and the surface hyphae, the latter usually more or less vertical and in some species forming a compact "palisade". Sporangia abundant on some agars, usually formed at the surface. Conidia formed in some species. On certain agars,

the mycelium of some species breaking up, when crushed, into irregular pieces of hyphae, rods and coccoid bodies.

Some isolates producing a distinctive pleasant or slightly unpleasant odor, others odorless.

Aerobic, Gram positive, acid-fast, and liquefying gelatin, so far as tested.

Saprophytic in a variety of soils and in fresh water, and world-wide in distribution.

The above description is based not only on the type species, *Actinoplanes philippinensis*, but on over a hundred and twenty isolates representing a number of species groups.

The genus is readily distinguished from *Streptosporangium*. On leaves, the latter produces a conspicuous aerial mycelium which resembles that in most species of *Streptomyces*, whereas, as a rule, no such mycelium is found in the isolates of *Actinoplanes*. As a whole, the isolates of *Actinoplanes* grow much more vigorously on agar than do those of *Streptosporangium*. The most striking difference is that in *Actinoplanes* the sporangiospores are motile, whereas in *Streptosporangium* they are non-motile.

Under certain conditions of culture, some species of *Actinoplanes* resemble some species of *Micromonospora*. Indeed, a nonsporangial strain of *Actinoplanes* might easily be confused with certain Micromonosporas. The spores of *Micromonospora*, however, are formed singly or in grape-like clusters but never in chains, whereas in *Actinoplanes* they are formed singly and also in chains but not in grape-like clusters. Furthermore, none of the collections of *Micromonospora* so far tested forms sporangia. Another striking difference is that, in most species of *Micromonospora*, on certain agars, the sporulating surface turns black, whereas this change does not occur in *Actinoplanes*. In general, the species of *Micromonospora* are less vigorous in growth than those of *Actinoplanes*.

Several species of *Actinoplanes*, when grown on potato dextrose and certain other agars, will form a small pasty culture which, when mounted and crushed under a coverslip, breaks up into minute spheres, irregular rods, and short-branched, hyphal segments, much as in *Nocardia*. Such growth, however, is not the normal condition for any species of *Actinoplanes*. None of the twenty-five species of *Nocardia* from Baarn and the American Type Culture Collection formed sporangia when grown either on any of the agars most favorable for sporangial formation or on *Paspalum* leaves in water.

SUMMARY

Streptosporangium is described as a new genus and the related genus *Actinoplanes* is revised. *Streptosporangium* is distinguished from *Actinoplanes* by having non-motile spores formed in sporangia, less vigorous growth on various agars, and greater development of aerial hyphae on leaves and on agar. The type species of the new genus is *Streptosporangium roseum*, which is characterized in part by the color of its aerial hyphae on potato dextrose agar. These two genera are so distinct from the other Actinomycetales that they are placed in a newly described family, the Actinosporangiaceae, the most outstanding characteristic of which is the formation of spores in sporangia.

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